

Senescence related alteration in hippocampal biogenic amines produces neuropsychological deficits in rats

Sadia Saleem, Saiqa Tabassum, Sana Ahmed, Tahira Perveen and Saida Haider

Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan

Abstract: Aging is a natural complex process that is regulated at genetic, cellular, molecular and systemic levels and leads to the development of a variety of changes including structural, chemical and genetic in the senescent brain. The major goal of the present study was to investigate the age associated cognitive dysfunction and other behavioral changes and their association with age related alterations in levels of neurotransmitters, such as dopamine (DA) and serotonin (5-HT) in the hippocampal region. Twelve male Albino Wistar rats were taken for the study including six young rats (04-05 months old) and six aged (20-22 months old) rats in each group. The learning and memory performance of rats was assessed by passive avoidance test (PA) and novel objective recognition task (NOR). Ambulatory activity was monitored by Open field test. Light/Dark transition test was used to monitor anxiety, whereas depression like symptoms was examined by Forced Swim Test (FST). Results showed that aged rats exhibited learning and memory impairment in PA and NOR. There was a negative relation between aging process and locomotion, consistent with previous findings. Moreover, an augmented increase in level of anxiety and depression was also observed in senescent rats. A marked decrease in DA and 5-HT was observed in the hippocampus of aged rats. Similarly, levels of 5-HIAA and DOPAC were also found to be decreased in aged rats. It is therefore concluded that age has a negative influence on cognitive function, depression, anxiety and locomotion in rats. Cells in all brain regions, especially hippocampus are affected by aging. In general aging exhibits a decline in sensory, motor and cognitive functions. These behavioral changes or functional deficits may be attributed to the age related decline in the levels of different neurotransmitters in brain /hippocampus. The present findings of behavioral deficits and altered neurotransmission in hippocampus of aged rats suggest a relationship between senescence, altered brain neurotransmitters and behavioral deficits.

Keywords: Senescence, depression, anxiety, cognition, 5-HT, DA

INTRODUCTION

Aging is any change in organism overtime or more simply the process of growing old which has positive as well as negative impact on functional skills of humans as well as animals. Some factors of aging grow and expand while others decline. Reaction time may slow down with age while knowledge and wisdom may expand (Unno and Hoshino, 2007). Evidence has shown that senescence of brain comprising diminishing cognitive and motor skills is a substantial socioeconomic problem (Hedden and Gabrieli, 2004). In almost all aged individuals the brain functions such as cognition and motor skills along with other organ systems are affected even in absence of distinct pathology. During senescence brain undergoes a series of changes that leads to shrinkage of brain and alteration in neuronal structure and function (Joseph *et al.*, 1995). These myriad of changes lead to several problems like incidence or risk of cancer, cardiovascular-related disorders (Unno and Hoshino, 2007), white matter lesions, depression, dementia and memory (Peters, 2006) and cognitive impairment that may distort the normal pattern of aging (Gunstad *et al.*, 2006) and may lead to changes in levels of neurotransmitters and hormones (Peters, 2006). Aging not only lead to synaptic loss but

also causes modifications in intracellular synaptic structures such as reduced number of mitochondria, synaptic vesicles, cisternae and presynaptic area that decreases the brain function and metabolism of synapses in the aged brain. Neuronal shrinkage in aging may be due to extensive neuronal loss in the senescent brain. Researchers have also reported the loss of dendrites and dendritic spines in the aged brain and these losses decrease the postsynaptic substrate for synaptic connection in the aged brains (Wong, 2002).

Existence of relationship between aging and neurochemical alterations in brain has been reported (Hegazy and Ali, 2011). Acetylcholine (Ach), DA and 5-HT are specifically involved in locomotion, mood disorders, learning and memory and other behavioral tasks. It has been found that with the increasing age dopaminergic pathways or dopamine levels itself decline, number of synapses/receptors are decreased or binding to receptors is decreased (Peters, 2006). Moreover, the levels of serotonin and brain derived neurotrophic factor are also reduces with advancing age that may be involved in the regulation of synaptic plasticity and neurogenesis (Mattson *et al.*, 2004). Number of other factors has also been reported to be involved in the brain senescence including calcium dysregulation (Toescu *et al.*, 2004),

*Corresponding author: e-mail: saida-h1@hotmail.com

generation of reactive oxygen species (ROS) (Melov *et al.*, 2004), mitochondrial dysfunction and impaired glucose metabolism (Nyberg *et al.*, 2004). Levels of growth hormone levels was also reduced with increasing age which may be linked to the cognitive performance of animals although the evidence is not clear (Sytze van Dam, 2004).

Evidences have shown that during senescence decline in cognitive and motor skills have been associated with several deleterious changes in the function and morphology of different brain regions that are normally associated with such functions (Forster *et al.*, 1996). However, the nature of the causal factors responsible for these deleterious changes is poorly understood (Forster *et al.*, 1996). Researchers have found out several factors such as alcohol abuse, chronic inflammation, vascular diseases and even stress, which influence the person's ability to think and remember as he ages. It has been reported that the core factors and mechanisms underlying senescence associated neurodegenerative diseases are very complex and mainly comprises both genetic and environmental factors (Unno and Hoshino, 2007).

Hippocampus is the most critical region of the brain and the numbers of neuronal processes in this brain area are particularly vulnerable to aging process. (Clark *et al.*, 2007; Macphail, 2002) Changes in plasticity in hippocampal networks have been reported earlier (Fadoo *et al.*, 2004). The objective of the present study was to investigate the neurochemical and associated neurobehavioral changes in aged rats. Since hippocampus is associated with learning and memory, the study was further aimed to specifically evaluate the neurochemical changes in hippocampus responsible for the cognitive decline in senescence.

MATERIALS AND METHODS

Animals

In the present study twelve locally bred Albino-Wistar rats were purchased from H.E.J. Institute of chemistry and divided into two groups, six young rats (04-05 months) and six aged rats (20-22 months). All animals were housed individually in separate cages under a 12 h light-dark cycle (lights on at 6:00h) and controlled room temperature (22±2°C) with free access to cubes of standard rodent diet and tap water for at least 3-4 days before experimentation. All experiments were conducted according to a protocol approved by Local Animal Care Ethical Committee.

Chemicals

All the chemicals used in the present study were of highest quality available.

Experimental protocol

The rats were assigned as young (04-05 month) and old (20-22 months) comprising six rats in each groups.

Behavioral tests performed were Passive Avoidance test and Novel Object Recognition test to assess memory performance, Open Field test to assess ambulatory activity, Light/Dark box test to assess anxiety and Forced Swim test was used to determine the depression like symptoms in rats. After behavioral testing, animals were decapitated and their brain was immediately removed from skull within 30 seconds. With the help of fine forceps the membrane covering the brain was removed and then using spatula brain was taken out and dipped in ice-cold saline and then placed in brain slicer to dissect out hippocampus. All samples were immediately freeze and stored at -70°C until the analysis of biogenic amines using HPLC-EC.

Behavioral analysis

Passive avoidance test

Passive avoidance apparatus comprises two equal compartments, an illuminated 'safe' and a dark 'punishable' one that are connected with the help of a door which allow free passage from one compartment to the another. Both compartments had a gridded floor consisting of rods having diameter of 5mm with 0.5cm distance between them. The test consists of two sessions; training session and testing session. In the training session the animal was placed in a lightened box. Once the rat entered into the dark compartment with its four paws it received a foot shock of 1.5 mA through the grid floor to its paws for 5 sec. After which it immediately came back to the lightened safe compartment. 24 hrs after the training period the testing was done. During the testing session the rat was placed in the bright compartment again for a maximum of 5 minutes and the step-through latency time that is the time elapsed before the rat entered the dark compartment was recorded with the help of stop-watch.

Novel object recognition task

For the novel object recognition task the apparatus used was an open arena of (50 × 50) with 42 cm high walls. In order to saturate it with olfactory stimuli, cleaning of box was not allowed throughout the experiment. The objects to be discriminated were two glasses filled with white cement (used as familiar objects) in order to make them heavy enough so that rats could not be able to move them, and a metallic colored object (used as a novel object). The size of the objects was 2.5 times the size of the rat so that the rat could easily sniff it. During the first training session individual rat was permitted to explore the open field arena for 10 min, so that the animal was familiarized to the environment. After a delay of 24 hrs second training session was performed. During this session in the open field arena two similar novel objects were placed and allow the animal to explore them for 10 min. After the delay of 24hrs the retention test was performed in which the animal was positioned back into the similar environment the only difference is that one of the familiar

objects (used in training session was now replaced by a novel object and each animal was given a maximum of 10 min to accumulate 30 seconds of object exploration (Rampon *et al.*, 2000).

Locomotor and exploratory activity

The locomotor activity of animals was monitored in the open field apparatus that consisted of a square area (76 × 76 cm) having 42 cm high walls. The floor of the arena was equally divided by the help of lines into 25 equal squares. To determine locomotor activity, the animal was placed in the central square of the open field arena and allowed to explore the area. The number of squares crossed by animal with its four paws was counted for 5 min as reported earlier (Haider *et al.*, 2004). Activities of control and test group were monitored in a balance design in order to avoid the order effect.

Light-dark box test

The light/dark box apparatus consists of two-compartment box out of which one box is brightly lighted (having transparent walls) and a dark (black) box, hence differing in their sensory properties. Both of the compartments are equal in size (26×26×26 cm each), with an access (12×12 cm) between them and both have lids over them which were kept closed during the conduction of experiment. During the test session the animal was positioned in the bright compartment of the box and allowed to move freely between the two compartments for 5 min (cut off time). The number of crossings made by each rat between the light and dark compartments of the box and the total time spent in each compartment was recorded (Blumstein and Crawley, 1983).

Forced swim test

The forced swim test apparatus was comprised of a glass tank having height of 56 cm and width of 30cm, which contained water at the height of 22 cm have 25°C temperature. In this glass tank animals were individually forced to swim for 6 minutes. The height of water was selected so that animal was prevented from touching the bottom of the glass tank and also to prevent its escape from the glass tank. The FST test is commonly used and standard pharmacological model for evaluating depression like symptoms/behavior in rats and mice (Porsolt *et al.*, 1978). When the rat was placed in an inescapable chamber which is filled with water then the development of the state of immobility reflect the cessation of persistent escape directed behavior. In the test session animal's swimming behavior was monitored which can be defined as movement throughout the swim chamber (glass tank). The immobility time (the latency time taken by rats to become immobile) was monitored. The animal is considered immobile when it makes no further attempts to escape and only tries to keep its head above the water.

Neurochemical Analysis

Frozen samples of hippocampus were homogenized in extraction medium using an electrical homogenizer and neurochemical analysis was performed to estimate the concentration of monoamines DA and 5-HT and their metabolites DOPAC and 5-HIAA in the rat hippocampus by HPLC-EC method as reported by Haider, *et al.* 2011. Detection of these biogenic amines in a single sample was done by using reversed phase HPLC with electrochemical detector at an operating potential of about +0.8V. A 5µ Shim-pack ODS separation column having internal diameter of 4.0 mm and length of 150 mm was used as the stationary phase. The mobile phase containing 0.023% octyl sodium sulphate (OSS) and 0.1M phosphate buffer of pH=2.9 was eluted through this column under high pressure of about 2000-3000 psi on Shimadzu LEC 6A detector. The resulting chromatograms showing positions of DOPAC, DA, 5-HIAA and 5-HT in sample and standard are given as fig. 9 (A & B).

STATISTICAL ANALYSIS

The data of behavioral and neurochemical analysis was analyzed by using Student's *t*-test and the values of $p < 0.05$ were considered as significant.

RESULTS

The results of passive avoidance test showed that ageing had a detrimental effect on the retention of rats which is presented in fig. 1. Retention of passive avoidance was assessed after 90 minutes of training. Student's *t*-test analysis revealed significant ($t=3.47$, $df=10$, $p < 0.01$) impairment in memory which was observed by a marked reduction in the latency time taken by aged rats to enter into the dark (punishable) compartment.

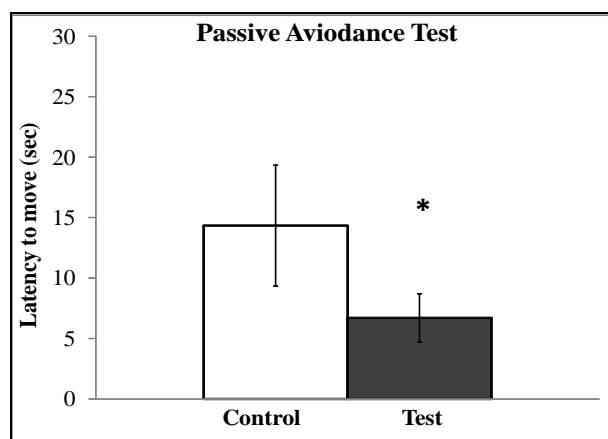


Fig. 1: Effect of age on the memory of rats tested in Passive Avoidance test. Values are means ± SD (n=6). Significant difference by Student's *t*-test; * $p < 0.05$ vs Control Group

The effect of senescence on object recognition of rats was shown in fig. 2 in terms of sniffing time for novel object. Young adults and aged rats were able to discriminate between novel and familiar objects, however the sniffing time for new object was significantly ($t=3.62$, $df=10$, $p<0.01$) lowered in old rats. Thus Student's *t*-test analysis showed age related object recognition impairment.

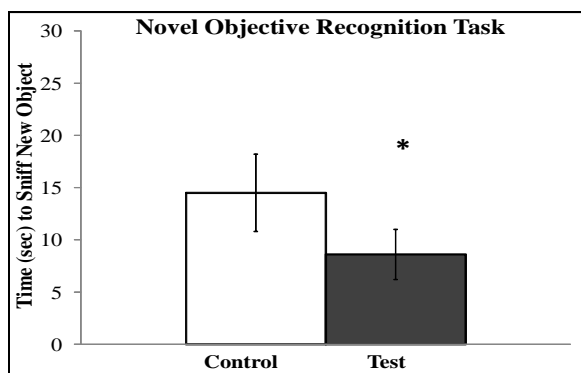


Fig. 2: Effect of age on the memory of rats tested in Novel Objective Recognition task. Values are means \pm SD ($n=6$). Significant difference by Student's *t*-test; * $p<0.05$ vs Control Group

Effect of ageing on ambulatory activity of rats was assessed in an open field arena which was presented in fig. 3. Student's *t*-test analysis showed a significant ($t=5.37$, $df=10$, $p<0.01$) decrease in the number of squares crossed by the aged rats as compared to young controls. These results indicate age-related hypoambulation.

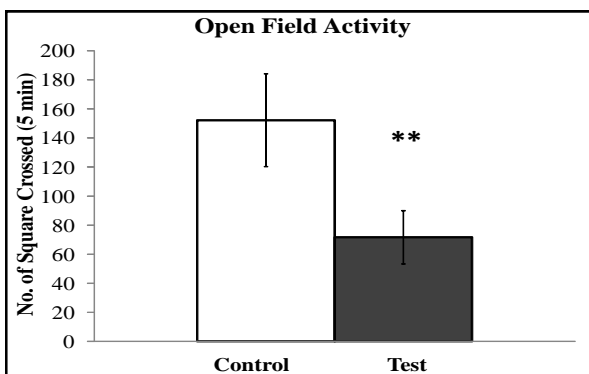


Fig. 3: Effect of age on ambulation of rats tested in an Open Field. Values are means \pm SD ($n=6$). Significant difference by Student's *t*-test; ** $p<0.01$ vs Control Group

Fig. 4 shows the age related changes in anxiety levels of rats in the light/dark box. Student's *t*-test analysis revealed a significant decline in the time spent by the aged rats in the light compartment relative to young controls. Statistical results of the data showed a significant ($t=7.93$, $df=10$, $p<0.01$) elevation of anxiety levels in aged animals relative to young adults.

Effect of ageing on depression in rats was determined by forced swim test which is shown in fig. 5. Analysis by

Student's *t*-test revealed a significant ($t=2.87$, $df=10$, $p<0.05$) increase in the immobility time in case of old rats as compared to young ones. These results revealed that depression was higher in aged rats relative to young subjects.

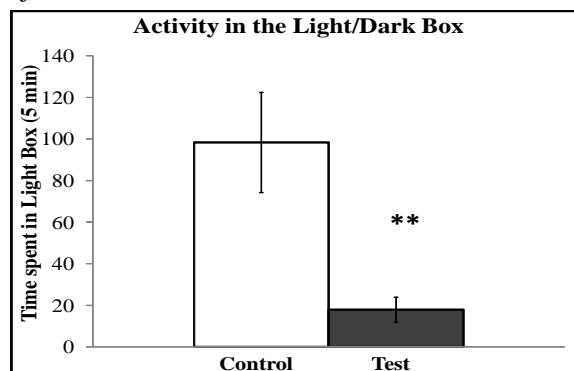


Fig. 4: Effect of age on anxiety in rats tested in the Light/Dark Box. Values are means \pm SD ($n=6$). Significant difference by Student's *t*-test; ** $p<0.01$ vs Control Group

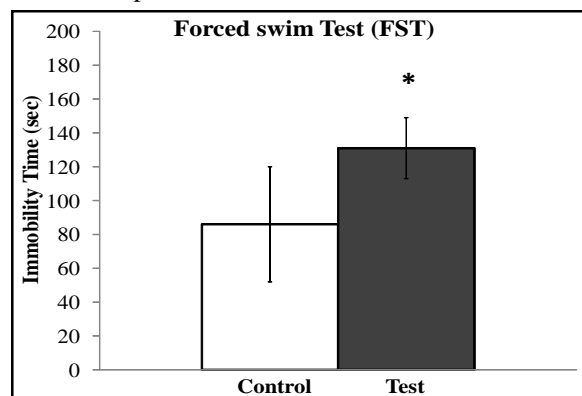


Fig. 5: Effect of age on depression in rats tested in Forced Swim test. Values are means \pm SD ($n=6$). Significant difference by Student's *t*-test; * $p<0.05$ vs Control Group

Fig. 6 (A & B) shows the age associated alterations in DA metabolism. Fig. A shows the changes in hippocampal DA levels of young and old rats. Analysis by Student's *t*-test showed a significant ($t=4.50$, $df=10$, $p<0.01$) decline in hippocampal DA levels of old rats relative to young controls. Fig. B shows a significant ($t=4.87$, $df=10$, $p<0.01$) decrement in the hippocampal DOPAC levels in senescent rats as compared to young control rats.

Fig. 7 (A & B) shows the effect of ageing on hippocampal 5-HT metabolism. Fig. A shows the effect of age on levels of 5-HT in the hippocampus of young and old animals. Analysis by Student's *t*-test showed a significant ($t=4.75$, $df=10$, $p<0.01$) decrement in the brain 5-HT levels of the old rats relative to young controls. Fig. B shows that the age induces a significant ($t=5.50$, $df=10$, $p<0.01$) decline in the levels of 5-HIAA in the hippocampus of rats.

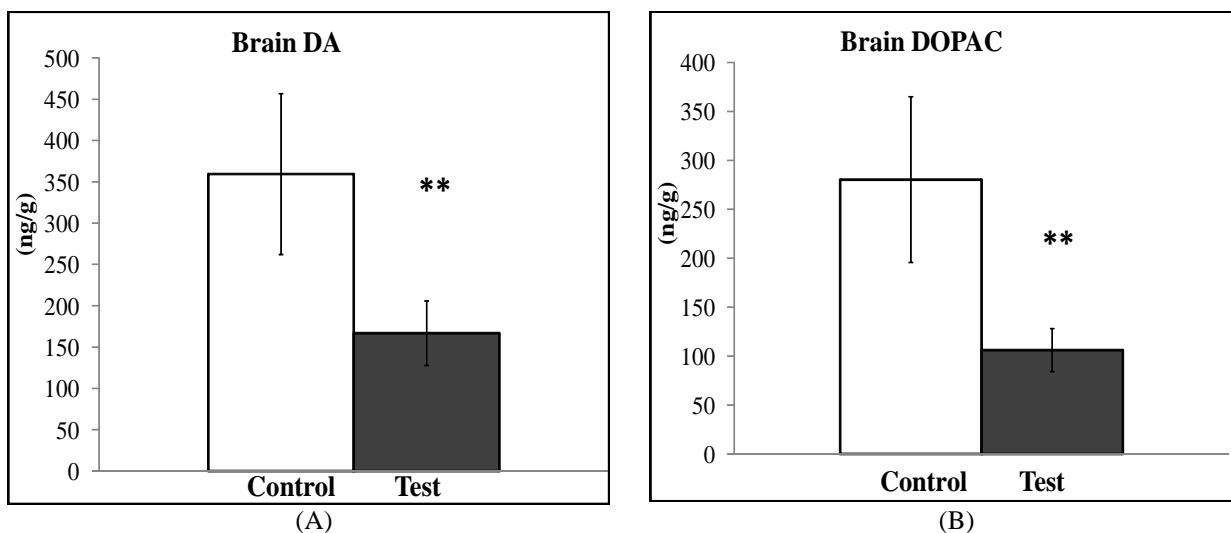


Fig. 6 (A & B): Effect of age on hippocampal DA and DOPAC levels in rats. Values are means \pm SD (n=6). Significant difference by Student's *t*-test; ** $p < 0.01$ vs Control Group

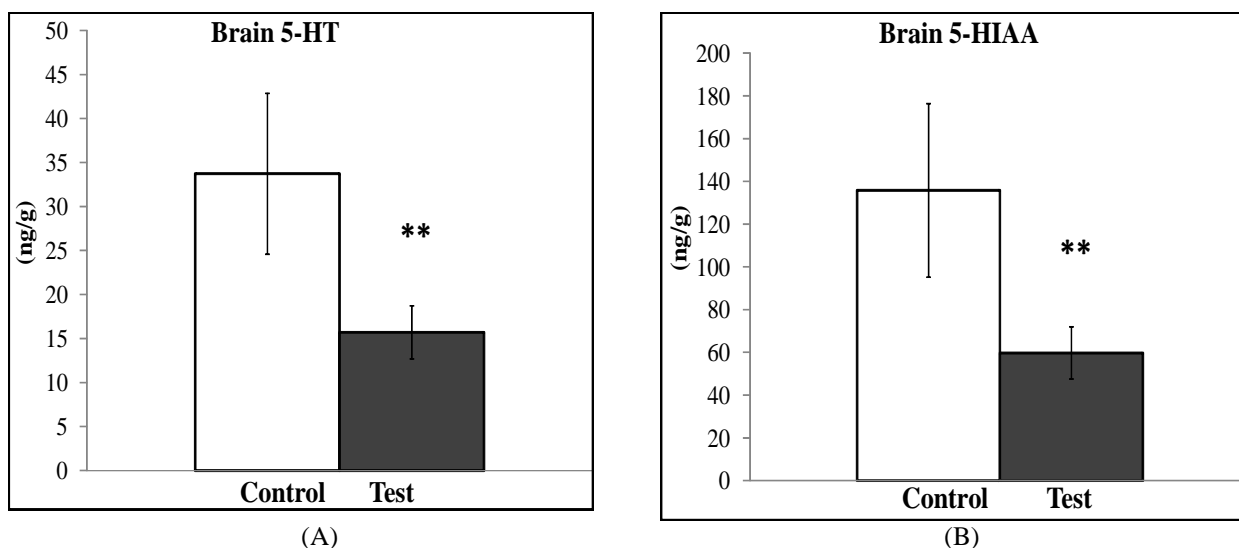


Fig. 7 (A & B): Effect of age on hippocampal 5-HT and 5-HIAA levels in rats. Values are means \pm SD (n=6). Significant difference by Student's *t*-test; ** $p < 0.01$ vs Control Group

Fig. 8 (A & B) show the effect of ageing on hippocampal 5-HT and DA turnover. Fig. A shows the effect of senescence on 5-HT turnover in the hippocampus of young and old animals. Analysis by Student's *t*-test showed a significant ($t=6.67$, $df=10$, $p < 0.01$) decrease in the hippocampal 5-HT turnover in old rats relative to young controls. Senescence also produces a significant ($t=2.99$, $df=10$, $p < 0.01$) decline in hippocampal DA turnover as shown in fig. B.

Fig. 9 (A & B) shows the chromatogram of standard solution and hippocampal samples. Fig. A shows the chromatogram of standard solution containing DA, DOPAC, 5-HT and 5-HIAA. Fig. B shows the chromatogram of biogenic amines in young and aged rats in the hippocampal region.

DISCUSSION

The process of aging or senescence is genetically programmed deterioration of all physiological functions with age. Several researchers reported the difference in parameters of behavior between senescent and young rats such as stamina, memory (Gerstein *et al.*, 2013), recognition (Renteria *et al.*, 2008) and depression (Oxenkrug and Rotner, 2012). However altered metabolism of neurotransmitters with age has been associated mainly with most of the age related neurodegenerative disorders. Age related structural and neurochemical changes are specific to the different brain regions (Mora *et al.*, 2007). The findings of the present study in aged rats revealed that hippocampal 5-HT and DA metabolism was significantly decreased. The turnover

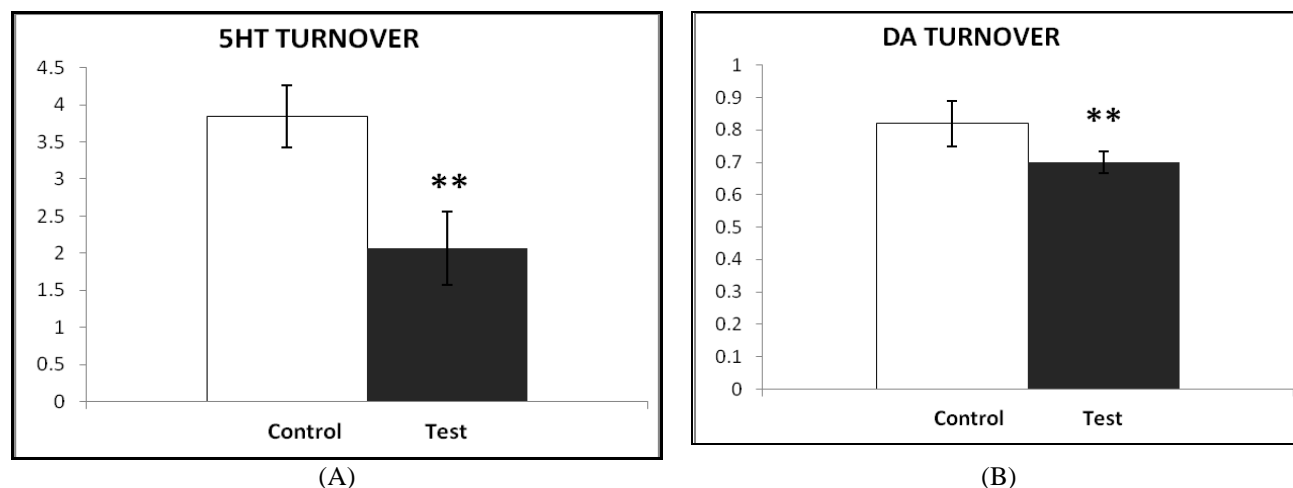


Fig. 8 (A & B): Effect of age on the levels of 5-HT and DA turnover in the hippocampus of rats. Values are presented as means \pm SD (n=6), significant differences by Student's *t*-test are shown as ** $p < 0.01$ with respect to controls.

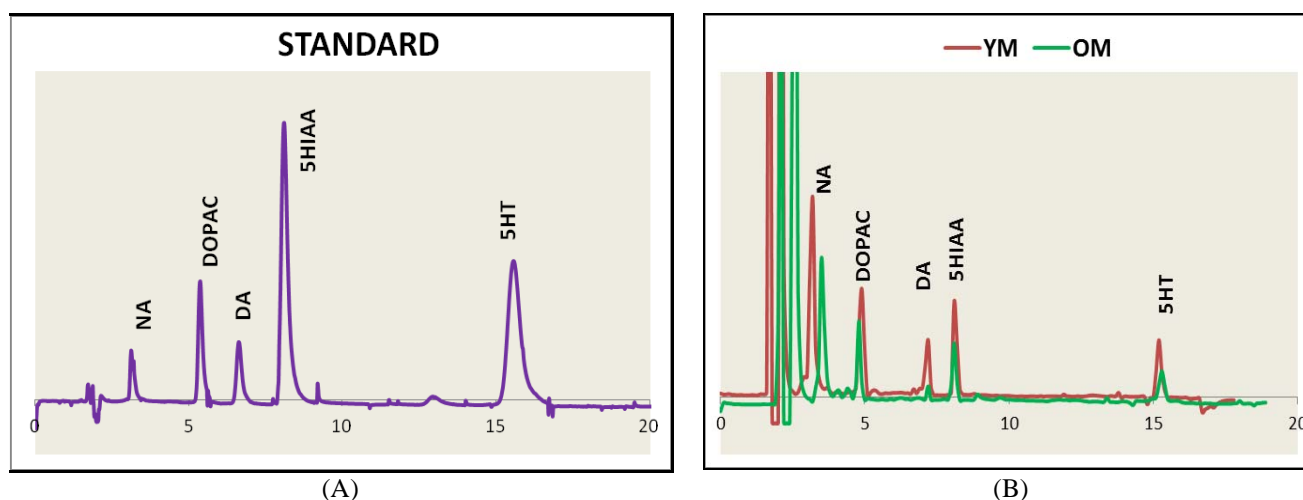


Fig. 9 (A & B): HPLC Chromatogram of Standard solution (A) and the biogenic amines in the hippocampal region of Young and Aged rats (B).

ratio of both neurotransmitters in aged rats was also decreased. The decreased in levels of both neurotransmitters indicates the existence of a reduction in the functional status of brain biogenic amines during aging. Experimental evidence supports the existence of a relationship between aging and various alterations of neurotransmitters in different areas of CNS (Ota *et al.*, 2006, Lee *et al.*, 2010). The decreases in neurotransmitters levels in aging have been attributed to either the degeneration of neurons with age or existence of a lower synthesis of neurotransmitters (Reis *et al.*, 1977, Míguez *et al.*, 1999).

Evidence shows that the older rats overall exhibited reduction in motor activity and other behaviors (Austin *et al.*, 1978). Simultaneous assessment of neurotransmitters and different behaviors in our study also shows a decline

in brain biogenic amines and associated behavior in aged rats. Senescent rats exhibited an impaired memory function, depression like symptoms and anxiogenic behavior. Multiple studies have shown alteration in monoamines and increased excitability in hippocampal neurons during aging (Hegazy and Ali, 2011). The behavioral deficits observed in present study may be attributed to the observed decreases in brain 5-HT and DA metabolism in aged rats. Both 5-HT and DA are implicated in various physiological functions such as memory (Meneses, 1999; Haider *et al.*, 2007; Nieoullon, 2002), depression (Celada *et al.*, 2004; Dunlop and Nemeroff, 2007), anxiety (Morelli *et al.*, 2011) and locomotor activity (Fletcher *et al.*, 2006). In the present study aged rats exhibited impairment in memory function and decline in cognitive ability. This impaired memory function may be related to the decreased 5-HT

metabolism in hippocampus of aged rats. The importance of hippocampus in spatial memory is known for decades (Clark *et al.*, 2007; Macphail, 2002). Patient with damaged to the hippocampal formation showed impairment in spatial memory (Lister and Barnes, 2009). Earlier reports also show that alterations in DA with age are associated with a decline in cognitive and motor performances (Peters, 2006). The present findings are in accordance with previous studies that throw light on age associated suppression of recognition memory in rats (Markham and Juraska, 2008).

DA also plays an important role in movement (Phillips *et al.*, 2008). A significant decrease in DA and its metabolites was observed in the hippocampus of aged rats compared to young rats. This decrease of DA metabolism may be associated to the age-related hypoambulation (Devid *et al.*, 1998, Altun *et al.*, 2007). The results of the present study demonstrate that aging impaired locomotion of the rats in the novel environment of open field arena, as old rats showed decreased ambulatory activity compared to young rats. Brain 5-HT levels are implicated in depression in humans (Cowen *et al.*, 2002) and animals (Haider *et al.*, 2013). In the present study aged rats exhibited a decrease in 5-HT and an increased immobility time in FST, which indicated depressive like behavior in these rats. Research on Senescence accelerated mice showed an increased level of depression along with the role of hippocampus (Onodera *et al.*, 2000). The age associated impairment in memory and depression like symptoms can be directly correlated with decreased levels of brain monoamines in senescent rats.

Evidence also shows a role of 5-HT in anxiety. An increase in 5-HT level has been implicated in anxiety like behavior (Kimura *et al.*, 2009). In the present study however decreased 5-HT levels and increased anxiety levels were exhibited by aged rats. It may be speculated that due to a persistent decrease in brain tissue level of 5-HT and 5-HIAA a compensatory mechanism may be upregulated in old rats. The anxiogenic receptors are concentrated in hippocampus, the brain region from where the anxiety signals generate and the dysfunctioning of hippocampus has been reported in old age (Burger *et al.*, 2007; Stoelzel *et al.*, 2002).

CONCLUSION

Earlier reports suggest that age affects neurotransmitter content in hippocampus which might increase the vulnerability of older individuals to the development of physiological and psychiatric disorders (Morgan and May, 1990). Senescence is the part of aging process that rats enter normally. In the present study neurochemical and neurobehavioral data together suggest that alteration specifically in hippocampal monoamines may be the cause of behavioral deficits observed in senescence.

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