Age-Related Decrease in Striatal DA Produces Cognitive Deficits in Male Rats

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Abstract: Aging is a process that presents various alterations in physiological, behavioral and neurochemical processes. It causes impairment of CNS functions which lead to changes in memory, cognition and other behavioral performances. Reports have shown that aging causes neurochemical alterations in various physiological functions. The aim of the present study was to evaluate the cognitive changes in relation to process of aging. For this 20 male rats were taken, 10 young (4-6 months) and 10 old (18-22 months). Morris Water Maze (MWM) test was performed to monitor changes in learning and memory while Object-Recognition Task (ORT) was performed to evaluate changes in cognitive function. After behavioral assessment decapitation was done and rat brain was dissected to isolate striatum. Then neurochemical analysis was performed by HPLC-EC to monitor changes in striatal DA and DOPAC levels. Results of behavioral tests showed that aged rats exhibited a significant impairment of long-term memory. While cognitive ability assessed by ORT was also impaired in aged rats. Neurochemical results showed that there was a significant decline in striatal dopamine (DA) concentration while its metabolite DOPAC was significantly increased in aged rats. Hence aging has a significant negative influence on cognitive functions. Age-related behavioral deficits may occur as a result of decline in DA levels in striatum leading to changes in memory and cognitive performance.

Keywords: Memory, cognition, aging, striatum, DA.

INTRODUCTION

Aging is the natural phenomenon, which is the process of growing old and is usually defined as the gradual biological impairment of normal function which has direct impact on the functional ability of organs and on the biological systems. These irreversible series of changes inevitably end in death. Evidence shows that physiological brain senescence, including declining cognitive and motor skills, is a significant socioeconomic problem that affects life quality of aged people [1]. Phenomenon of aging leads to changes in the brain size, vasculature, and cognition, therefore as age increases the brain shrinks and changes occur from the level of molecules to morphology [2].

The aging brain undergoes a myriad of changes resulting in alterations in neuronal structure and functioning that ultimately leads to degeneration of cognitive ability [3] which is the most widely acknowledged psychological change with age [4]. Reports have shown that the debilitating consequences of aging which include delayed amnesia and impairment of learning and memory are the major cognitive deficits [5, 6] and other deficits are decreased learning ability, slower thinking, and memory impairment. It is evident that various cognitive performances declined in aging that include processing speed [7] and episodic memory [8, 9]. Declining cognitive functions in aging is a complex process that starts to become obvious during middle age in humans (35–65 years old) and rats (12–24 months old) even in the absence of any neurodegenerative disease [10].

Research shows that striatum is an important brain region to determine biological correlates of age-associated cognitive alterations [9]. It is evident that striatum is a key structure for processing motivationally relevant events [11]. The human striatum shrinks with age and the rate of shrinkage varies among the striatal components [12]. It is also reported that DA receptor-containing neurons may be more vulnerable than other types of striatal neurons [3]. The striatum has been implicated in perceptual and cognitive speed [13] and loss of striatal DA receptors is one of most robust and functionally important neurochemical markers of aging [3, 14]. Declines in striatal volume of older adults [15] and in DA D_1 and D_2 receptors availability [16, 17] suggests that the learning and motivational functions of the striatum may be effected [11]. It is reported that reward and punishment processing might be disrupted in the aging striatum [11].

It is evident that age-related decline in brain DA activity is associated with reduction in cognitive and motor performance [16]. It is also reported that 10% per decade decrease in DA levels from early adulthood is

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associated with decreased cognitive and motor ability [2, 18, 19]. Reports have shown that as the age increases, the dopaminergic pathways between the frontal cortex and the striatum were reduced, or the level of DA decline, synapses/receptors are decreased or receptor binding is declined [2, 18]. It has been documented that D_1 receptor [17] and the dopamine transporter [20] are also reduced in aging. With the increasing age serotonin and brain derived neurotrophic factor levels fall that could be involved in the adult brain neurogenesis and regulation of synaptic plasticity [21]. Degeneration of the neurons receiving dopaminergic projections from the *Substantia nigra* has been suggested to be another possible cause of striatal shrinkage [12]. In addition, decreased concentration and decreased synthesis of DA receptor mRNA has also been associated with aging [3].

The aim of present study was to examine the relationship among striatal DA, aging and cognitive functions. Previous studies and results have shown a decrease in brain neurotransmitters in advanced age or adulthood. The present study was further aimed to investigate the consequences of aging on learning and memory functions and cognitive ability in aged rats and their relationship with striatal DA levels.

MATERIALS AND METHODS

Animals

Twenty healthy male Albino Wistar rats, in two different age groups were purchased from Animal House of HEJ Research Institute of Chemistry, University of Karachi, Pakistan. One group was assigned as “Young Controls (4-6 months)” rats weighing 100-150 grams and “Aged (18-22 months) rats” weighing 300-350 grams. The animals were caged separately in plastic cages with open access to tap water and cubes of standard rodent diet during the whole session of experiments. The room temperature was maintained at 22±2°C. Before starting the each experimental session, rats were accustomed to various handling procedures in order to avoid any stress effect. The familiarization was performed in order to nullify the psychological affliction of the environment. All experimentations in this study were accomplished according to the protocol recommended by Local Animal Care Ethical Committee.

Experimental Protocol

Rats were divided into two groups, Control and Test. Young rats (4-6 months) were assigned as Control and Old-age rats (18-22 months) were assigned as Test. After familiarization the behavioral tests were performed that includes Object-Recognition Task (ORT) and Morris Water Maze Test (MWM). Rats were then decapitated and their brain was removed within 30 seconds from skull. The membrane covering the brain was removed with the help of fine forceps. The brain then taken out using spatula was dipped in ice-cold saline and then positioned in brain slicer to dissect out striatum. All samples were stored at -70°C until analysis of biogenic amines by HPLC-EC. Frozen striatum samples were homogenized in extraction medium using an electrical homogenizer and neurochemical analysis was performed to estimate DA and DOPAC concentrations in brain. Estimation of DA and its metabolite DOPAC in the rat striatum was done by HPLC-EC method as reported by Haider, *et al.*, 2004 [24]. These biogenic amines were detected in a single sample by reversed phase HPLC with electrochemical detector at an operating potential of +0.8. A 5µ Shim-pack ODS separation column of 4.0mm internal diameter and 150mm length was used as the stationary phase. The mobile phase consisting of 0.023% octyl sodium sulphate (OSS) 0.1 m phosphate buffer at pH=2.9 was passed through this column under high pressure of 2000-3000 psi on Schimadzu LEC 6A detector.

BEHAVIORAL ANALYSIS

Morris Water Maze Test (MWM)

Morris Water Maze (MWM) test was performed to examine the effects on spatial memory. It was developed by Richard G. Morris in 1981 [22]. This is a well-known, conventional cognitive test which requires an animal to use spatial learning and memory to locate a hidden platform just below the surface of a circular pool of water and also to remember its location as in the previous trial [23]. It is reported that the animal uses cues in order to locate the hidden platform. The maze used for rats is same as described by Srikumar *et al.*, with some modifications [24]. It is a circular pool of water with a diameter of 45cm, height 37cm and depth of water is 12cm. The pool is a metal cylinder painted white on the inner surface and the escape platform is also made of metal cylinder with flat metallic top having a surface diameter of 8cm and is 2cm below the surface of water during water maze training. The pool is filled with water (23 ± 2ºC) and made opaque with milk in order to obscure the platform and to allow proficient tracking of the swim paths of the rats (Figure **1**). In our experiment we have assessed the reference

Figure 1: Morris Water Maze Test Apparatus used to assess learning and memory performance in rats.

(long-term) memory and working (short-term) memory in terms of latency to locate the escape platform. The test is based upon 2 phases; the training phase and the test phase. Memory functions of rats were tested by noting down the retention latency. The cut off time was 2 minutes for each session. Initially the training session was performed during which each rat was placed into the water in such a way that their face was towards the wall of the tank. After placing 120 seconds were given each animal to find and mount onto the hidden platform, if the rat located the platform it was allowed to stay on it for 10 seconds. If it failed to locate the platform during the allocated time then it was guided gently onto the platform [26].. Then test was performed which is further divided to two trials; STM (Short Term Memory) and LTM (Long Term Memory). Interval between trials was important. STM was assessed 60-90 minutes after training session and LTM was measured after 24 hours.

Object Recognition Task

The novel object recognition test was used to assess the cognitive ability of animals. It was established by Ennaceur and Delacour in 1988 to assess the rat's ability to distinguish a novel (new) object in a familiar environment [25]. This test is used to assess memory for interactions with novel objects. This is a test of recognition memory. The procedure performed in this experiment was same as described by Okuda which comprised of exposing rats to two similar objects and then recording their discriminating ability to discriminate the novel object placed at the same time along with one of familiar objects [28]. In this test the method used was essentially the same as that Okuda et al 2004 with slight modifications. The apparatus consists of square box made of gray painted wood having dimensions of $45x45x45cm^3$ (Figure **2**). The objects to be discriminated were two similar transparent glasses filled with white cement (A1 and A2) and a metallic container of same size filled with white cement (new object, B). The test was performed in three phases; habituation, training, and test session. On day 1, rats were undergone pre-exposure to habituate them to the testing chamber for 10 minutes. Next day 24 hrs after pre-exposure rats was positioned inside the box with two similar objects for 10 minutes. On third day, the test phase was performed during which animal is exposed to one of the known object and a novel object, for 3 minutes. In the test phase the sniffing time for the novel and familiar object was measured. Reports have shown that no difference in exploration for two objects at the test phase can be interpreted as a memory deficit [26]. Discrimination index, the difference in exploration time of familiar and novel objects, was calculated to evaluate cognitive performance as described by Okuda *et al.*, [28].

Figure 2: Object Recognition Task Apparatus used to determine the discriminative power and cognitive ability in rats.

Statistical Analysis

Results are represented as means ± S.D. Statistical analysis was performed by Students *t*-test. Values $p<0.05$ were considered as significant.

RESULTS

Figures **3a** and **3b** show the effect of aging upon short-term memory and long-term memory in young and aged rats. Analysis by *t*-test showed that in aged rats there was no effect on short-term memory compared to control rats however long-term memory was impaired significantly in old rats which was evident by a significant ($p<0.01$) increase in the latency time. A 65 % increase in latency time was exhibited by aged rats compared to controls.

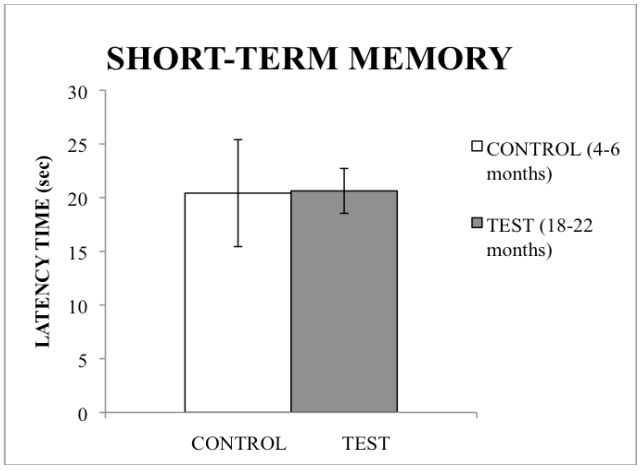

Figure 3a: Effect of aging on STM in rats. Values are the means ± SD (n=10). No significant difference by Student's *t*-test Vs control rats.

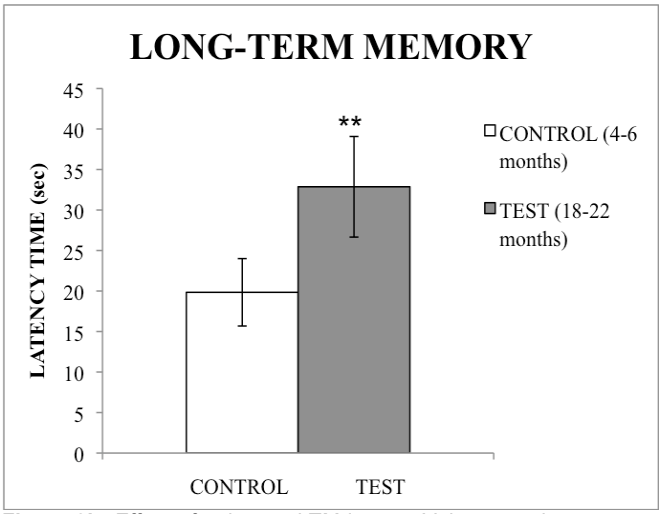

Figure 3b: Effect of aging on LTM in rats. Values are the means ± SD (n=10). Significant difference by Student's *t*-test, **$p<0.01$ Vs control rats.

Figure **4a** and **4b** shows the effect of aging upon cognition in rats, assessed by Novel object recognition (NOR) Test. On comparison of sniffing time for both objects (old and new) by both groups (young and aged) results revealed a significant ($p<0.01$) decline in sniffing time for new object by aged rats as compared

to young controls while sniffing time for old object showed no significant difference (Figure **4a**). Discrimination index was calculated by comparing difference in sniffing time for new and old object and analyzed by Students *t*-test. Analysis by *t*-test revealed a significant ($p<0.01$) decline in discrimination index as sniffing time for a new object in aged rats was decreased. A significant (26 %) decrease in sniffing time for new object and a significant (32 %) decrease in the discrimination index indicated an impairment of cognition in aged rats.

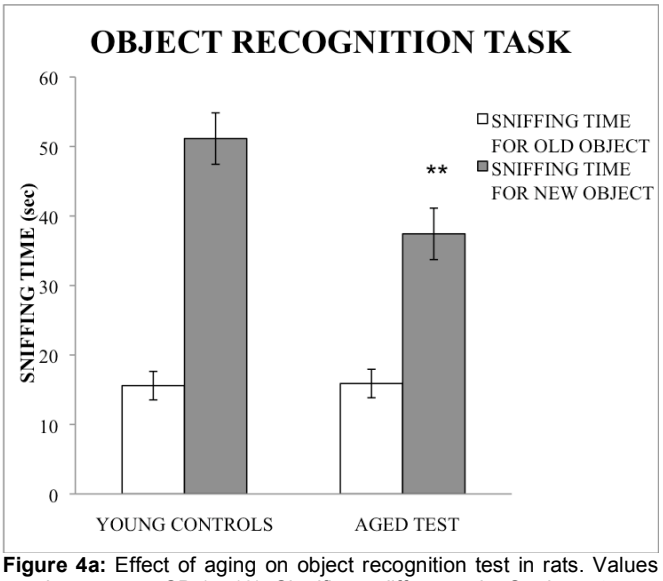

Figure 4a: Effect of aging on object recognition test in rats. Values are the means ± SD (n=10). Significant difference by Students *t*- test, **p<0.01 for sniffing time of new object by aged rats compared to young controls

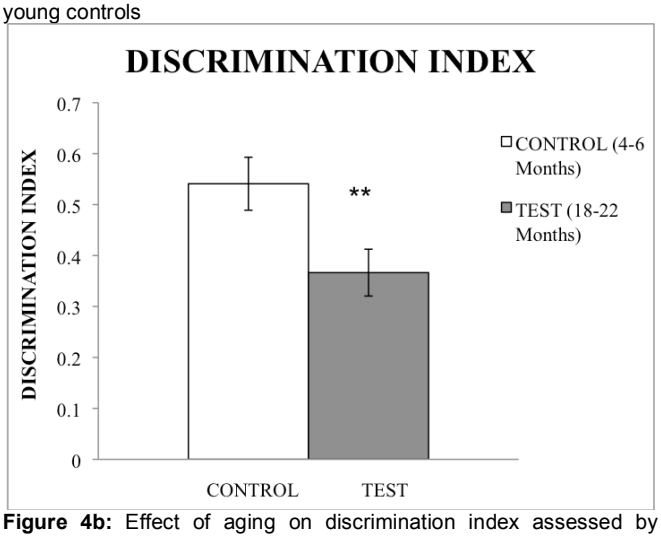

Figure 4b: Effect of aging on discrimination index assessed by object recognition task in rats. Values are the means ± SD (n=10). Significant difference by Students *t*- test, **p<0.01 Vs control rats.

Figure **5a** and **5b** show the effect of aging upon striatal DA and DOPAC levels in rats. Analysis by *t*-test revealed a significant ($p<0.01$) decline in striatal DA levels in aged rats while DOPAC levels in the striatum of aged rats were increased significantly ($p<0.01$). Aged rats exhibited a 29% decrease in striatal DA and a 57% increase in striatal DOPAC levels.

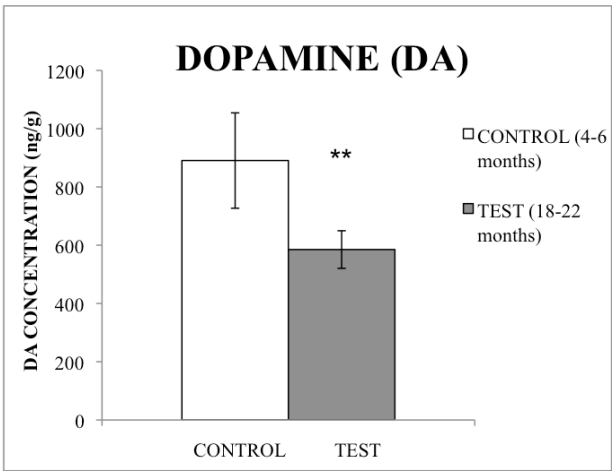

Figure 5a: Effect of aging upon striatum dopamine concentration in rats. Values are the means ± SD (n=10). Significant difference by Students *t*-test, **p<0.01Vs control rats.

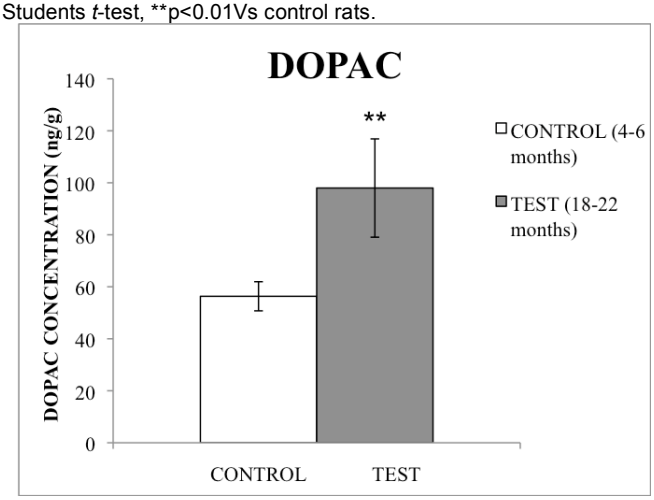

Figure 5b: Effect of aging upon striatum DOPAC concentration in rats. Values are the means ± SD (n=10). Significant difference by Students *t*-test, **p<0.01Vs control rats.

DISCUSSION

Aging is one of the major aspects of human life and has both positive and negative effects on functional abilities of the human being as well as animals. Evidences show that normal aging is related to a decline in size and number of neurons, loss of synapses and neuronal branching and to the decreased functioning of neurotransmitter systems specifically the dopamenergic and serotonergic system and these structural and functional changes have particularly significant impact on the individual's behavioral, cognitive and affective status [27]. With aging a number of processes are affected such as memory, learning and other cognitive abilities such as; thought process, abilities to activate and focus

attention. It is also reported that cognitive impairment in rodents, like other species is a consequence of advancing chronological age. Various reports have shown that as compared to young rats, aged rats perform worse on a broad range of learning and memory tasks [28-30]. Extensive research has focused on understanding that the definite aspects of observed aging deficits, in animal models of cognitive aging [31-34], were mediated due to alterations in the brain systems. The behavioral assessment of cognitive ability in rodent models of aging presented a basis for understanding biological factors that are responsible for these impairments.

In the current study both behavioral and neuropharmacological effects of normal aging were examined. The rats of two different age groups young and aged rats were compared and cognitive behavioral parameters such as short-term memory, long-term memory and recognition memory were assessed. DA and DOPAC concentration in striatum were also assayed.

The present results showed no significant effect of aging on STM while LTM was found to be impaired in aged rats. Aged rats displayed spatial memory impairment which was evident by increased latency time to locate the submerged platform. It has been suggested earlier that the aged rats utilize non-spatial strategies to unravel the task, and thus displayed learning and memory impairment. The present finding showed a significant decrement in memory and cognitive behavior with concomitant decline in striatal DA levels in aged rats. Oxidative damage has also been believed as a possible cause of age-related brain dysfunction since the brain is supposed to be principally susceptible to oxidative stress owing to a comparatively high rate of oxygen free radical generation [35-38]. It is also reported that neuronal and cognitive dysfunction may also occur due to oxidative damage to brain mitochondria, protein, and nucleic acid [39]. Therefore, the impaired memory function exhibited by the aged rats in the current study might be due to oxidative damage to the brain.

In the present study cognitive impairment associated with aging was monitored by the object recognition test. The results showed a significant decrease in the sniffing time for new object, indicating a cognitive impairment in aged rats. These findings are in agreement with the previous studies which showed that 24 hour after training aged rats exhibited impaired recognition memory retention compared to young animals [40]. The effects of age on brain neurotransmitters vary in different brain systems and regions and are often controversial. The present study was aimed to examine the age-related neurotransmitter alterations. To further elucidate the association, the concentrations of DA and its metabolite DOPAC were simultaneously assayed in the striatum region reported to have significant role in cognition [9]. The neurochemical analysis illustrated a significant increment in the turnover of DA. However the concentration of DA was significantly decreased in striatum of aged rats. This decrease in DA could be accountable for the observed cognitive deficits in the present study. While increased DA turnover in present study may be attributed to increased activity of MAO-B in aged rats reported earlier [41]. Moreover inhibition of MAO has been shown to increase DA concentration and hence memory function.

CONCLUSION

Aging has a significant effect on both behavioral and neurochemical aspects of life. From the current study it is concluded that aged rats show a significant decline in cognitive performance compared to young rats and this decline in cognitive performance may be directly related to decreased striatal dopamine concentration.

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