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Neuronal Function in Male Sprague Dawley Rats During Normal Ageing

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Summary: During normal ageing, there are physiological changes especially in high energy demanding tissues including the brain and skeletal muscles. Ageing may disrupt homeostasis and allow tissue vulnerability to disease. To establish an appropriate animal model which is readily available and will be useful to test therapeutic strategies during normal ageing, we applied behavioral approaches to study age-related changes in memory and motor function as a basis for neuronal function in ageing in male Sprague Dawley rats. 3 months, n=5; 6 months, n=5 and 18 months, n=5 male Sprague Dawley Rats were tested using the Novel Object Recognition Task (NORT) and the Elevated plus Maze (EPM) Test. Data was analyzed by ANOVA and the Newman-Keuls post hoc test. The results showed an age-related gradual decline in exploratory behavior and locomotor activity with increasing age in 3 months, 6 months and 18 months old rats, although the values were not statistically significant, but grooming activity significantly increased with increasing age, $p<0.05$. Importantly, we established a novel finding that the minimum distance from the novel object was statistically significant between 3 months and 18 months old rats, ($p<0.05$) and this may be an index for age-related memory impairment in the NORT. Altogether, we conclude that the male Sprague Dawley rat show age-related changes in neuronal function and may be a useful model for carrying out investigations into the mechanisms involved in normal ageing.

Keywords*:* Neuronal function; male Sprague Dawley rats, Ageing

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INTRODUCTION

Normal ageing comes with cell dysfunctions that are not pathological (Lister and Barnes, 2009). Among affected tissues are brain and skeletal muscle. It was once thought that loss of neurons contributes to the events leading to loss of function in the nervous system. However, imaging and quantification studies show no loss of neurons in the ageing brain (Rapp and Gallagher, 1996; Rasmussen et al., 1996; Rapp et. al., 2002). The human population has witnessed an increase in the number of ageing population in the last century owing to the advancement in medicine, science and technology (U.S. Bureau of the Census *1996; Pollack, 2005)***.** The mean life expectancy has also increased significantly in the last few decades (Lunenfeld, 2008). With the continuous increase in the number of ageing population, it is important to study the changes that occur with normal ageing, the mechanisms of these changes and find therapeutic strategies that can be applied to preserve function with increasing age. It is believed that during normal ageing subtle cellular changes occur leading to diminished function, loss of function and vulnerability to disease (Li et al. 2013, Martinelli et al., 2013). The reasons for these changes are still being elucidated with various experimental approaches and findings (Li et al., 2013). There are about nine theories that have been given by different scientists as the reasons why humans and other species age (Gershon and Gershon, 2000; Jin, 2010). This has made the understanding of the ageing process a daunting task and has challenged researchers to answer the present questions using different approaches. In order to find the underlying mechanisms and study therapeutic targets or strategies that can be applied during age-related dysfunction, there is a need to establish the characteristic age-related changes present in the animal model to be used and the unique parameters that were compromised during ageing. In this study, we carried out an assessment of neuronal function using established neurobehavioural approaches in male Sprague Dawley rats in order to identify the functional changes that occur during normal ageing. Neurobehavioural assessments were used as indices for neuronal function and behaviour scored include head-dipping and grooming (Brown et. al., 1999; Podhorna and Brown, 2002). Rearing and head-dipping show the animals' exploratory capacity while grooming is regarded as a displacement behavior and a consequence of a neuronal dysfunction or disorder (Jolles et. al., 1979; Greer and Capecchi, 2002). It is believed that with

much testing using appropriate models and standardized methods, the complex mechanisms involved in the normal ageing process will be unraveled. Ultimately, it should help discover therapeutic targets and strategies that will help boost function and improve the quality of life among the increasing ageing population.

MATERIALS AND METHODS

Animals

Fifteen male Sprague dawley rats were raised and nursed in the animal house facility of the Lagos State University College of Medicine under conditions approved by the University Ethics Committee. They were fed ad libitum with rat chow and water. The animals were grouped as follows: 3 months; n=5, 6 months; n=5 and 18 months old; n=5, male Sprague Dawley rats. The animals were exposed to 12 hour light and 12 hour dark daily cycle.

Neurobehavioral Assessments

We carried out extensive assessment of neuronal function using the Novel Object Recognition Task, NORT (Ennaceur and Delacour, 1988) and the Elevated Plus Maze, EPM (Lister, 1987). The parameters studied were indices of neuronal functional integrity across the ages of animals studied. Briefly, the NORT was developed in 1988 by Ennaceur and Delacour. The NORT box (120cmX40cm) was made of wood and plexi-glass. The box is divided into three compartments (40cmX40cm) and a square hole measuring (10cmX10cm) allowed the animal to move freely within the compartments. In the first stage, two identical objects were kept in the two opposite compartments of the box and the animal is allowed to investigate the objects. In the second stage, a new (novel) object is introduced into one of the opposite compartment and the animal is introduced into the box for 5 minutes. Parameters such as the speed of the animal, total distance travelled, time investigating the novel object, minimum distance from the novel object were measured using the ANY-maze behavioural tracking software (Stoelting Co., USA) attached to an overhead camera. When introducing each animal and at every stage, the box was cleaned with 70% alcohol to remove olfactory cues. An advantage of the NORT compared with other methods is that it is less stressful for the animals which may affect their memory function (Sik et al., 2003). The Elevated Plus Maze (EPM) Box was built according to the description by Lister 1987. The box is made up of clear plexi-glass and ply-wood which forms a plus. The Plus Maze is elevated from the ground by 60cm. The base is made of ply wood. The open arms (50cm X 10cm) of the maze are made of ply wood with an elevation of about 2cm. In the closed arms (50cm X 10cm), a clear plexi-glass 50cm X 40cm is fixed at two adjacent sides. This allows the observer to view the behavior of the animal when it is on this end of the box. A ply wood of 40cm X 10cm closes up the closed arm of the elevated plus maze. Animal behavior assessment was carried in diffused light conditions using the ANY-maze behavioral tracking software (Stoelting Co., USA) connected to an overhead Logitech camera at the center of the room. In both EPM and NORT, testing was carried out under diffuse lighting.

Statistical Analysis

Results are presented as means ±S.E.M. Statistical analysis was carried out using ANOVA followed by Newman-Keuls post-hoc test on the ANY-maze behavioral tracking software.

RESULTS

Novel Object Recognition Task

In the Novel Object Recognition Task, 18 months old rats show a decline in learning and memory by showing changes in their interaction around the novel object. (Figure 1 and 2). In particular, the minimum distance from the novel object was statistically significant ($p \leq 0.05$) across the groups, the oldest rats having the farthest distance from the novel object (Figure 1). The minimum distance from the novel

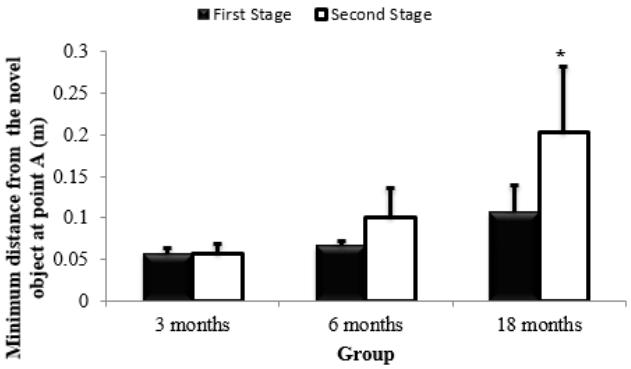

Fig. 1: Minimum distance from the center point (point A) of the novel object as a measure of memory function Two-way ANOVA with one repeated measure (Stage)Treatment F (2, 12) = 4.6227; p=0.031. Post-hoc analysis Factor – treatment 3 months old versus 18 months old ^{q} (24, 3) =3.6248; *p=0.044

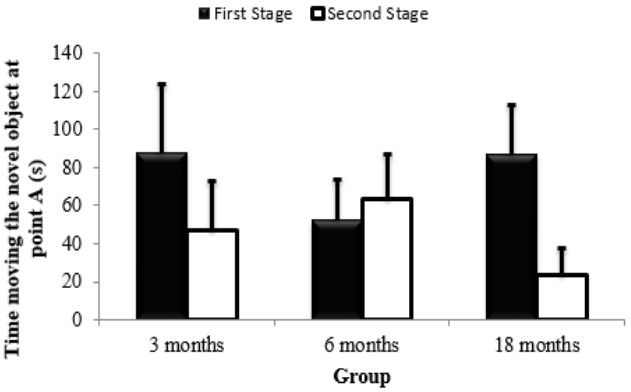

Fig. 2: Time moving towards the novel point A as a measure of memory function Two way ANOVA with one repeated measure (Stage) F (2, 12) = 0.1236; p=0.885

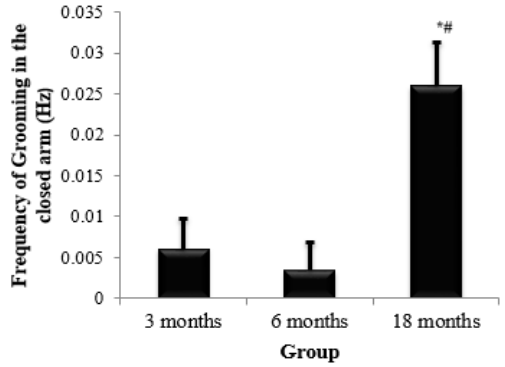

Fig. 3: The frequency of grooming in closed arm of the EPM increase with age. One way ANOVA: F $(_{2, 12})$ = 8.4548; p=0.005 * p<0.006 (3 months vs 18 months); #p<0.007 (6 months vs 18 months).

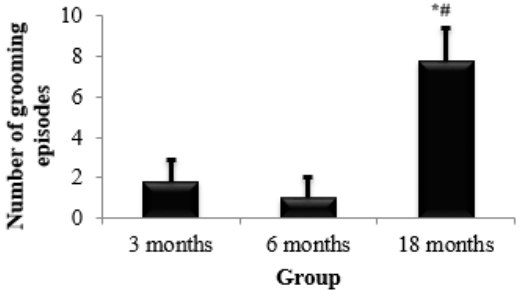

Fig. 4: The number of grooming episodes in the closed arm of the EPM increased Significantly in 18 months old animals One way ANOVA: F $(_{2, 12})$ = 8.6695; p=0.005 *p<0.006 (3 months old versus 18 months old); #p<0.007 (6 months old versus 18 months old).

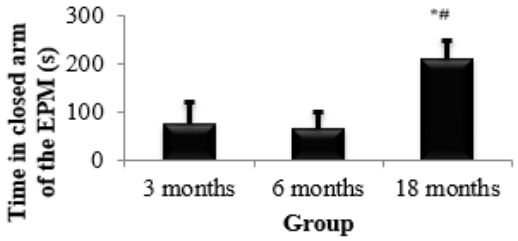

Fig. 5: Time in closed arm of the maze as a measure of non-exploration of the EPM. One way ANOVA: F $(_{2, 12})$ = 4.4149; p=0.037.* p<0.028 (3 months old vs 18 months old); #P<0.027 (6 months old vs 18 months old).

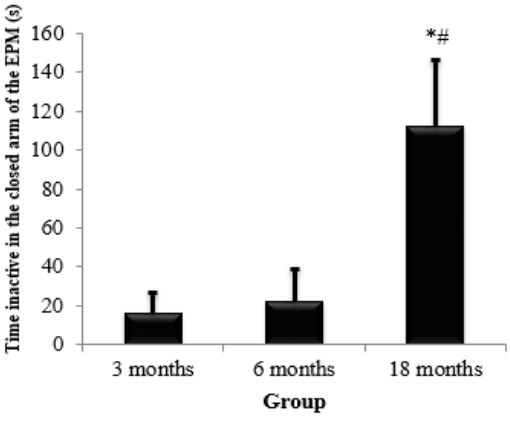

Fig. 6: Time inactive in the closed arm of the EPM as a measure of motor function. One way ANOVA: F $(_{2, 12})$ =5.6329; p=0.019 *p=0.028 (3 months versus 18 months) #p=0.016 (6 months versus 18 months).

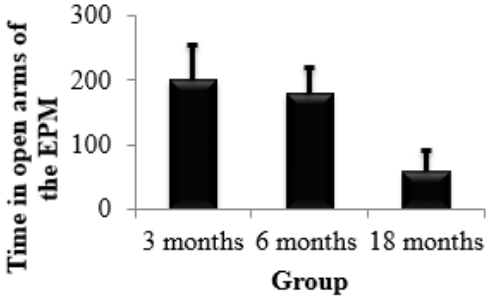

Fig 7: Time in the open arm of the EPM. One Way ANOVA: F (2, 12) =3.0730; p= 0.084

object is here-in reported for the first time in literature as a possible index for memory dysfunction in the Novel Object Recognition Task.

Elevated Plus Maze Task

The results of the Elevated Plus Maze Task showed an age dependent increase in the episodes of grooming in the closed arm in 3 months, 6 months and 18 months old animals. The frequency and number of grooming episodes was significantly raised in 18 months old rats compared with 3 months and 6 months old rats, $p \leq 0.05$ (Figure 3 and Figure 4). Furthermore, time spent in the closed arm of the Elevated Plus Maze which implies non-exploration was significantly raised in 18 months old animals compared with 3 months and 6 months old animals, $p \leq 0.05$ (Figure 5). Similarly, the time of inactivity in the closed arm of the Elevated Plus Maze was significantly raised in the 18 months old rats compared with 3 months and 6 months old rats respectively, $p \leq 0.05$ (Figure 6). Finally, the mean time spent in the open arms of the Elevated Plus Maze reduced with increasing age, although the values were not significant, $p \geq 0.05$ (Figure 7).

Furthermore, 3 months old rats had the highest number of entry into the open arms of the Elevated Plus Maze, which implies increased exploration in the 3 months old.

DISCUSSION

In the Novel Object Recognition Task (NORT) aspect of this study, a new finding reported is the minimum distance from the novel object which was significant across the all the age groups. The young animals showed a closer interaction with the novel object compared with the older animals. Basically, the NORT is a measure of changes in the time interacting with the novel object compared with a familiar object as an index of memory function (Ennaceur and Delacour, 1988; Brown et al., 1999; Podhorna and Brown, 2002). We suggest that the minimum distance from the novel object may be an important parameter in the NORT to assess memory function and that the changes observed in the animals are evidences of neuronal dysfunction occurring during ageing.

Grooming behaviour in rodents can be a response to a stressful stimulus, a sign of frustration or an unexpected stimulus (Fentress 1968a, b,). Although rodents normally groom for approximately 15-20% of the total duration of a behavioural test, excessive grooming can be a sign of a central nervous dysfunction (Graybiel and Saka, 2002; Greer and Capecchi, 2002). Our findings in the Elevated Plus Maze Task (EPMT) are consistent with these reports in the percentage time spent grooming in the 3 months and 6 months old Sprague Dawley rats. However, the grooming episodes and frequency of grooming increased significantly in the 18 months old rats (Fig. 3 and Fig. 4), showing a rise in grooming behaviour during old age. Herein we report that the excessive grooming observed in the old animals reflects a sign of changes in the central nervous system function due to ageing. It is believed that significant increase in the number and frequency of grooming behaviour observed in the oldest rats may be a consequence diminishing neuronal function when animals were presented to a new environment.

Finally, the higher open arm activity observed in the younger rats shows enhanced motor function and exploratory behaviour of the young animals over the older 18 months old in the EPM. Open arm entries observed in the rats were not significant across the groups; however, they show a gradual decline with increasing age. Furthermore, the higher percentage of time spent in the closed arm of the EPM by the oldest animals implied an increased anxiety-like behaviour in the group (Fig. 5 and Fig. 6). This is consistent with the report of Pellow et al., 1985; Pellow and File, 1986, and Brett and Pratt, 1990, that anxiogenics will reduce the time spent in the opened arm of the EPM. We report that these observations are basically age-related, and a consequence of an underlying subtle changes in neuronal function. While the underlying mechanisms are still being investigated and evaluated, our study further showed that the Sprague Dawley rat strain is an appropriate animal model that can be used to investigate the mechanisms underlying age-related neuronal dysfunction occurring during normal ageing.

We concluded that the male Sprague Dawley rat show peculiar changes in neurobehavioural function during normal ageing hence is a useful animal model for future studies that will elucidate the underlying mechanisms that drive these changes or to test for possible therapeutic targets. It is recommended that the ages of this strain of rodents be taken into consideration when using this animal model for investigations involving central nervous system function, as ageing may alter neuronal function.

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REFERENCES

Brett, R.R. and Pratt, J.A. (1990). Chronic handling modifies the anxiolytic effect of diazepam in the elevated plus-maze. European Journal of Pharmacology. 178:135-138.

Brown, R. E., Corey, S. C., Moore, A. K. (1999). Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behavior Genetics, 26, 263-271.

Ennaceur, A., Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behavioural Brain Research 31:47-59.

Fentress, J.C. (1968a). Interrupted ongoing behavior in two species of vale (Microtus agretis and Clethyrionomys brittannicus) I. Response as a function of preceding activity and the context of apparently irrelevant motor pattern. Animal Behaviour, 16, 135-153.

Fentress, J.C. (1968b). Interrupted ongoing behavior in two species of vale (Microtus agretis and Clethrionomys brittannicus) II: Extended analysis of motivational variables underlying fleeing and grooming behavior. Animal Behaviour, 16, 154-167.

Gershon, H. and Gershon, D. (2000). Paradigms in ageing research: A critical review and assessment. Mechanisms of Ageing and Development 117: 21–28.

Graybiel, A.M. and Saka, E. (2002). A genetic basis for obsessive grooming. Neuron. 3;33(1)1-2.

Greer, J.M. and Capecchi, M.R. (2002). Hoxb8 is required for normal grooming behaviour in mice. Neuron 3;33(1)23-34.

Jin, K. (2010). Modern Biological Theories of Aging. Aging Dis. 1(2):72-74

Jolles, J., Rompa-Barendregt, J., Gispen, W.H. (1979). Novelty and Grooming Behavior in the Rat. Behavioral and Neural Biology 25, 563-572.

Li, H., Sharma, L., Li, Y., Hu, P., Idowu, A., Liu, D., Lu, J., and Bai, Y. (2013). Comparative bioenergetic study of neuronal and muscle mitochondria during aging. Free Rad. Biol. Med., 63, 30-40.

Lister, J.P., Barnes, C.A. (2009). Neurobiological changes in the hippocampus during normative ageing. Arch. Neurol. 66(7):829-833.

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology, 92: 180-185.

Lunenfield B. (2008). An Aging World – demographics and challenges. Gynecological Endocrinology 24(1): 1-3.

Martinelli, P., Sperduti, M., Devauchelle, A.D., Kalenzaga, S., Gallarda, T., Lion, S., Delhommeau, M., Anssens, A., Amado, I., Meder, J.F., Amado, I., Meder, J.F., Krebs, O.O., Oppenheim, C., Piolino, P. (2013). Age-Related Changes in the Functional Network Underlying Specific and General

Autobiographical Memory Retrieval: A Pivotal Role for the Anterior Cingulate Cortex. PLoS ONE 8(12):1-11.

Pellow, S., Chopin, P., File, S.E., Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of neuroscience methods; 14 (3): 149–167.

Pellow, S. and File, S.E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacology Biochemistry and Behavior:(24);525-29.

Podhorna, J., Brown, R. E. (2002). Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. Genes, Brain and Behavior. 1, 96-110.

Pollack M.E. (2005). Intelligent Technology for an Aging Population. AI Mag. 26 (2): 9-24.

Rapp, P.R., Deroche, P.S., Mao, Y., Burwell, R.D. (2002). Neuron number in the parahippocampal region is preserved in aged rats with spatial learning deficits. 12(11):1171-9

Rapp, P.R., Gallagher, M. (1996). Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. Proc Nat Acad Sci USA:93 (18):9926-30.

Rasmussen, T., Schileman, T., Sorensen, J.C., Zimmer, R.J., West, M.J. (1996). Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. Neurobiol. Aging. 17(1):143-7.

Sik, A., van Nieuwehuyzen, P., Prickaerts, J., Blokland, A. (2003). Performance of different mouse strains in an object recognition task. Behavioural Brain Research 147:49-54

US Bureau of the Census 1996