J. Afr. Ass. Physiol. Sci. 3 (1): 36-40, July 2015.

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

http://www.jaaps.aapsnet.org

Research Article

The therapeutic potential of melatonin on neuronal function during normal ageing in male rats

A.J. Idowu and I. Olatunji-Bello

Department of Physiology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.

Keywords:
Melatonin, neuronal function, motor function, normal ageing

ABSTRACT

Background: Ageing is a common factor in the onset neuronal dysfunction and neurodegenerative diseases. Serum level of melatonin (N-acetyl-5-methoxytryptamine), a free radical scavenger, reduces significantly during normal ageing and in neurodegeneration. **Methods:** To test the therapeutic potential of melatonin on function during normal ageing, we carried out an assessment of neuronal function in six months, nine months, twelve months and twenty-four months old male Sprague Dawley (SD) rats with 0.1mg/kg exogenous melatonin. Data was analyzed using one way analysis of variance (ANOVA) and Student-Knewman Keuls post-hoc test. **Results**: In six months old SD rats, melatonin treatment for two months restored motor function in the Chimney test. Furthermore, melatonin administration improved exploratory behavior and motor activity in ageing rats in the Elevated plus Maze (EPM) task. Finally, only 33% of melatonin treated rats had died at the termination of the experiment while all controls had 100% mortality. **Conclusion**: Melatonin may be a beneficial therapeutic agent to improve neuronal function during normal ageing.

INTRODUCTION

Normal ageing often leads to the decline in cell function and the onset of major degenerative diseases. In particular the brain and skeletal muscle are affected leading to reduced function and susceptibility to age-related disease. There has been a search for therapeutic targets to boost function during normal ageing. Among such proposed compounds is melatonin- (N-acetyl-5-methoxytryptamine). Serum melatonin levels decrease with increasing age (Waldhauser et al., 1988). Experiments in mice observed an extension of life span with administration of melatonin but suggested replicative studies to justify the findings (Pierpaoli and Regelson, 1994). Melatonin administration also increased life span in fruit fly (Bovilla et al., 2002). However, the available evidence of the role of melatonin has not validated its therapeutic use convincingly (Karasek, 2007). Uniquely among many compounds, melatonin has no known LD50 and it is nearly non-toxic (Gitto et. al., 2012). Given that ageing affects the entire human population with a corresponding rise in the number of age-related disease, it is important to look into possible therapeutic agent that will preserve function during ageing. Currently, available data and conflicting reports have not allowed for the approval of melatonin as a remedy for age-related cell dysfunction. Hence, this study further investigates the therapeutic potential of melatonin in improving neuronal function during ageing.

Address for correspondence:
E-mail: abimbola.idowu@lasucom.edu.ng

METHODS

Animals

Twenty-seven (27) male Sprague Dawley Rats were used for this study. The animals were raised in the animal house of the Lagos State University College of Medicine, Lagos, Nigeria. Institutional guidelines for the care, use and handling of animals were followed. Animals had 12-hour light and 12-hour darkness and were fed with rat chow and water *ad libitum.*

The Elevated plus Maze (EPM) Task

Locomotor activity and exploratory behavior was studied using the Elevated plus Maze Task (Lister, 1987; Brown et al., 1999). The Elevated plus Maze (EPM) box was built according the description of Lister, 1987 with some modifications in the dimension to allow for the size of adult and aged rats. The Elevated plus Maze Box was made from clear plexi-

glass and ply-wood which forms a plus. The Plus Maze was elevated from the ground by 60cm. The open arms (50cm X 10cm) of the maze were made of ply wood with an elevation of about 2cm. In the closed arms (50cm X 10cm), clear plexi-glass 50cm X 40cm was fixed at two adjacent sides. This allowed 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 closed up the closed arm of the elevated plus maze. Behavioural assessment was carried out in diffused lighting using the Any Maze behavioural tracking system connected to an overhead camera. Animals were introduced into the EPM box for five minutes and behavioural parameters were measured or scored. The EPM box was cleaned with 70% ethanol before introducing the next animal to remove any olfactory cues.

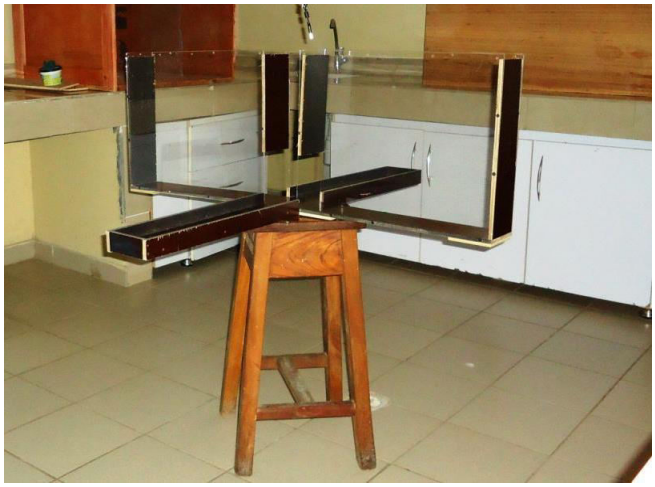

Fig. 1:
The Elevated Plus Maze Box

Chimney Test

Motor function was assessed using the Chimney Test. The Chimney test was first described by Boissier and colleagues in 1960. The inability of an animal to climb backward up through a plastic or glass tube within a given period of time is regarded as an indication of neurologic impairment (Boissier et. al., 1960) and this was used to assess motor function in rodent animals. The chimney comprises a glass tube, diameter of 44mm and length 320mm in length. Rats were introduced into the glass tube after placing the tube in a horizontal position and allowing the rats to walk into the tube. When the animal reaches the end of the tube, the tube is inverted or turned vertically so that the animal is in an upside-down position. This triggers an escape response and the animal starts to climb backwards up the tube. Using a stop watch, the time it took for the rats to climb out was measured (3min cut off time and 6 trials for each animal).

Drug administration

Melatonin (0.1mg/kg) was administered subcutaneously to animals late evening for eight weeks. Melatonin (Sigma Aldrich) was freshly prepared for daily administration. Melatonin (Sigma Aldrich) was dissolved in 10% ethanolic saline before administration.

Statistical Analysis

Data was plotted as mean + standard error of mean. Statistical analysis was carried out using one way ANOVA followed by Student-Knewman Keuls post-hoc test on the ANYMAZE Software behavioral tracking system. Student t-test (unpaired) was used where appropriate.

RESULTS

Total Measures of Activity in the Elevated plus Maze (EPM) task

The overall measure of activity in the two zones, A and B (i.e. arms) in the Elevated plus Maze (EPM) task is shown in fig. 2a, 2b, 2c, and 2d. Melatonin administration increased total distance covered and average speed in the EPM box in all age groups (Fig. 2a and 2b), although these changes were not significant. Similarly, the total number of grooming episodes was reduced in 24 months old rats by melatonin treatment (Fig. 2c). In contrast, we found a significant increase in the duration of head dip behaviour in melatonin treated 24 months old rats compared with untreated controls of the same age (Fig. 2d).

Zone A (Closed arm) activity measures in the EPM task

The time spent in the closed arm of the EPM box was shorter in melatonin treated 24 months old rats (Fig. 3a). The mean average speed in the closed arm was increased with melatonin treatment, although these parameters were not statistically significant (Fig. 3a and 3b). The time inactive in the closed arm and the number of grooming episodes varied directly with the total time spent in the closed arm in all the age groups (Fig. 3c and 3d).

Zone B (Open arm) activity measures in the EPM task

In 9 months and 12 months old rats, melatonin treatment did not have any significant effect on the distance covered in the open arm of the EPM box, however, in the 24 months old rats, melatonin administration significantly raised distance covered and the number of head dips in the open arms (Fig. 4a and 4b). Similarly, the number of grooming episodes and the frequency of grooming was significantly raised in the 24 months old melatonin treated rats compared with untreated controls of the same age (Fig. 4c and 4d).

Chimney Test

In the Chimney test, melatonin significantly improved motor function in 6 months old rats which received melatonin treatment for eight (8) weeks compared with controls (Fig. 5).

Longevity Studies

At the termination of the experiment, 100% death was recorded in the ethanolic saline treated 24 months old rats group, while only 33% death was recorded in the melatonin treated 24 months old rats group (Fig. 6).

DISCUSSION

Our findings show that there were improvements in motor function and activity in the melatonin treated 24 months old rats which were not significant (Fig. 2a, 2b,

2c). Early melatonin intervention before rats reach old age is therefore recommended for future studies. In addition, closed arm (Zone A) activity observed in the EPM task showed that melatonin has a potential to alter function favourably as ageing sets in (Fig. 3a, 3b, 3c,

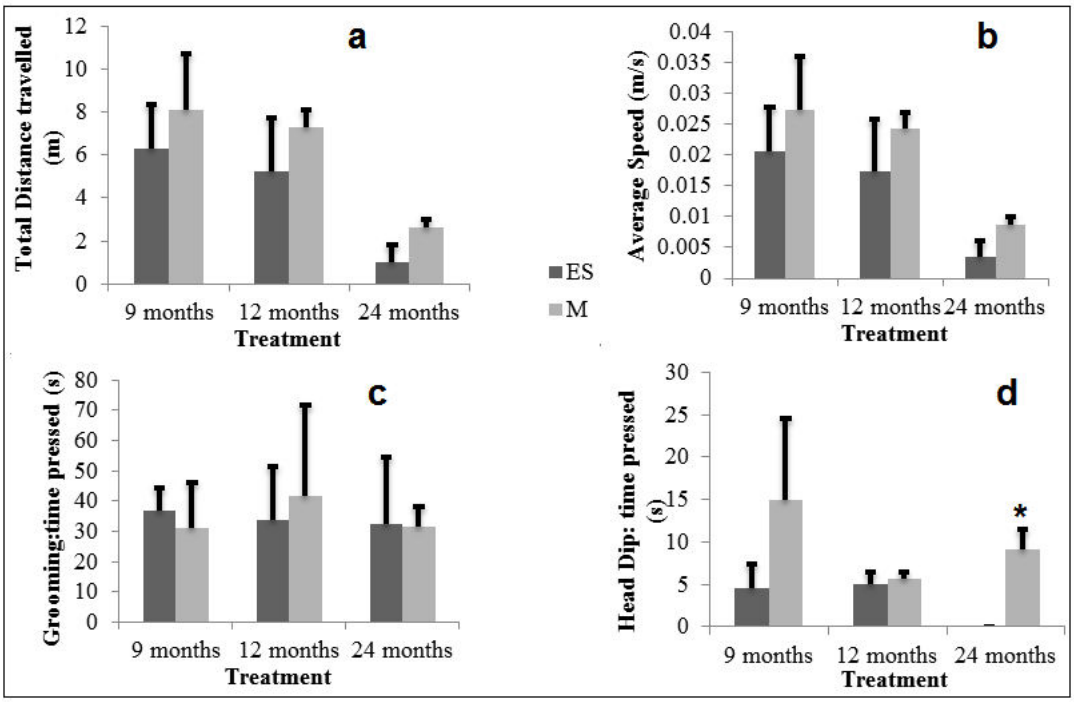

Fig. 2: (a) Total distance travelled in the EPM in ethanolic saline (ES) and melatonin (M) treated rats. ANOVA F $(_{5, 11})$ = 1.9044; p>0.05. (b) Average speed in the EPM in ethanolic saline (ES) and melatonin (M) treated rats with age. ANOVA F $(_{5, 11})$ = 1.8891; p>0.05. (c) Time spent grooming in the EPM in ethanolic saline (ES) and melatonin (M) treated rats. ANOVA F $(_{5, 11})$ = 0.0501; p>0.05. (d) Total time of head dips in the EPM in ethanolic saline (ES) and melatonin (M) treated rats. ANOVA F $(_{5, 11})$ = 1.1480; p>0.05. *p<0.05; 24 months (ES) vs 24 months M

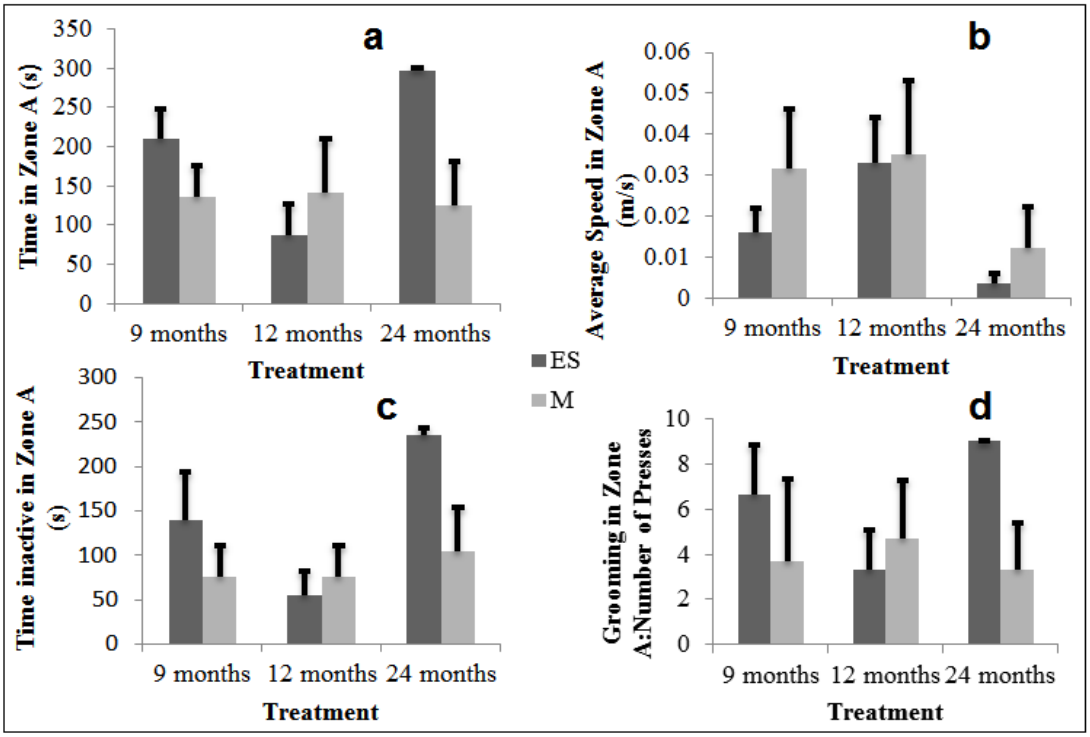

Fig. 3: (a) Time spent in the Zone A, the closed arm of the EPM in ethanolic saline (ES) and melatonin (M) treated rats. ANOVA F (5, 11) = 1.9639; p>0.05.(b) Average speed in the closed arm of the EPM in ethanolic saline and melatonin treated rats. F (5, 11) = 1.0137; p>0.05 (c) Time inactive in the closed arm of the EPM in ethanolic saline (ES) and melatonin (M) treated rats. F $(_{5, 11})$ = 2.1273; p>0.05 (d) Grooming in the closed arm of the EPM in ethanolic saline (ES) and melatonin (M) treated rats. F (5, 11) = 0.7104; p>0.05

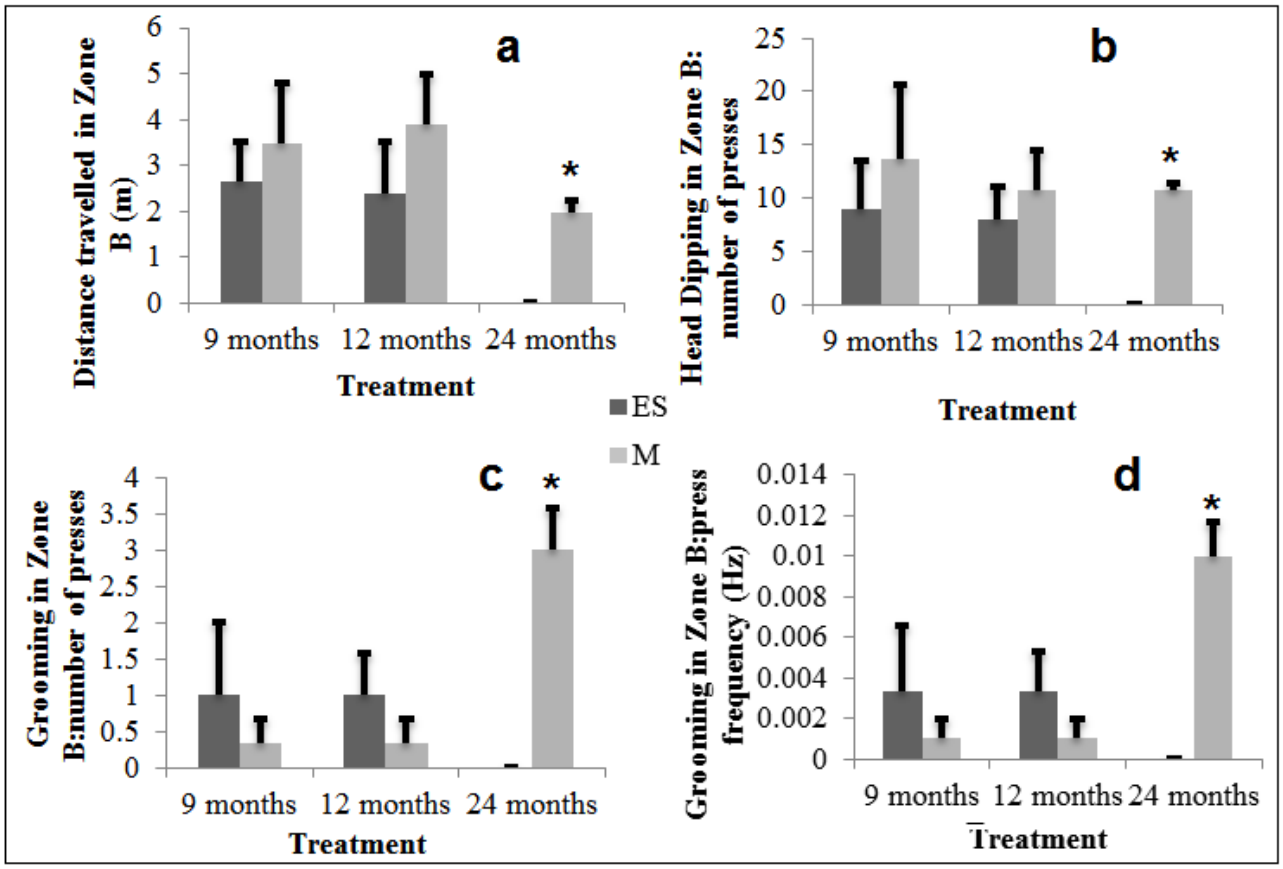

Fig. 4: (a) Distance travelled in the open arm of the EPM in ethanolic saline (ES) and melatonin (M) treated rats. ANOVA F $(_{5, 11})$ = 1.6593; p>0.05. *p<0.05; 24 months (ES) vs 24 months (M). **(b)** Number of Head dips in the open arm of the EPM in ethanolic saline (ES) and melatonin (M) treated rats. ANOVA F $(_{5, 11})$ = 0.9717; p>0.05 *p<0.05; 24 months (ES) vs 24 months (M). **(c)** Number of grooming episodes in the open arm of the EPM in ethanolic saline (ES) and elatonin (M) treated rats. ANOVA F $(_{5, 11})$ =3.2353; *p<0.05 *p<0.05; 24 months (ES) vs 24 months (M) **(d)** Frequency of grooming in the open arm of the EPM in ethanolic (ES) and melatonin (M) treated rats. ANOVA F $(_{5, 11})$ = 3.4123;* p<0.05. *p<0.05; 24 months (ES) vs 24 months (M)

3d). We hypothesize that at old age during normal ageing, the total restoration of normal function using melatonin treatment may depend on the integrity of melatonin receptors. Convincingly, melatonin receptors have been found to mediate some of the therapeutic actions of melatonin (Dragicevic et al., 2011, Wang et al., 2011) and in the absence of the receptors, melatonin may not be able to restore or improve function during old age (Dragicevic, 2011).

Furthermore, open arm activity was improved in melatonin treated 24 months old animals compared with untreated controls, Fig. 4a, 4b, 4c, 4d. Head-dip behavior, a characteristic feature of explorative behavior in the open arms of the EPM box (Brown et al., 2009) was significantly raised in melatonin treated 24 months old rats (Fig.4b). Although grooming behavior in rodents is considered a displacement behavior (Espejo et al., 1997), excessive grooming may be a sign of genetic neurological disorder according to Greer and Capecchi, 2002. The results show a reduction in excessive grooming in the closed arm of the EPM box in melatonin treated 24 months old rats (Fig. 3d). Interestingly, melatonin treated 24 months old rats' show grooming behavior in the open arm of the EPM box (Fig. 4c and 4d). This grooming behavior seen in the open arm of the EPM box in this group is within the normal grooming limit of about 10 – 15% of the mean total time spent by rodents when placed in a novel environment as reported by Jolles et al., 1979. In this study, melatonin sustained normal grooming (Fig. 2b) but prevented excessive grooming which is characteristic of neuronal dysfunction or nervous disorder.

In the chimney test, melatonin restored motor function in 6 months old treated male Sprague Dawley rats which received melatonin for eight weeks (Fig. 5). This improvement seen with melatonin treatment is an indication of an age-related downturn in normal function in the animals and this is consistent with an earlier report that the decline in motor function occurs

before old age (Li et al., 2013). Herein, we report that melatonin carries out its positive influences on neuronal function effectively during the early stages of ageing. This may account for the inability of melatonin to significantly improve or preserve some motor functions in 24 months old rats in the EPM task (Fig. 2a, 2b).

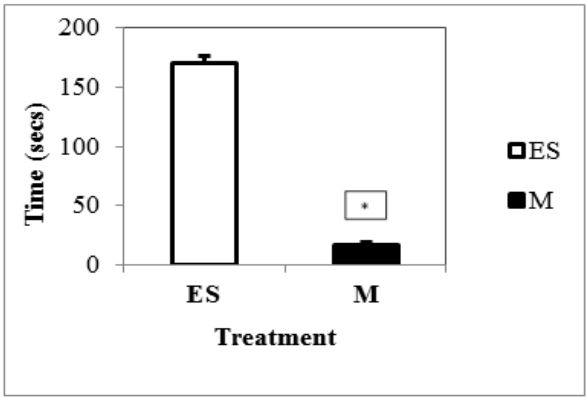

Fig. 5: Chimney test in ethanolic Saline (ES) treated and melatonin (M) treated animals in male Sprague Dawley rats, *p<0.05.

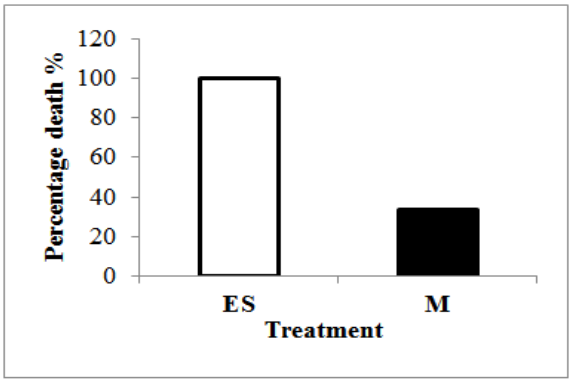

Fig. 6: Percentage death in 24 months old male Sprague Dawley rats after ethanolic saline (ES) and melatonin (M) treatment for eight weeks.

Finally, our longevity studies with exogenous melatonin administration show an increase in life span in the melatonin treated old rats. At the time of concluding the experiments after melatonin administration, we recorded 100% death in the 24 months old ethanolic saline treated rats while only 33% death was recorded in the 24 months old melatonin treated rats (Fig. 6). Although there are conflicting reports on the ability of melatonin to extend life span (Pierpaoli and Regelson, 1994; Karasek, 2007), this study has contributed to the available data on the role of melatonin in life span extension. Hence, we conclude that, melatonin is able to improve neuronal function when administered early and that melatonin may be a potent therapeutic agent during normal ageing.

ACKNOWLEDGEMENT

We acknowledge the support of the Lagos State University College of Medicine for providing funding for the research project.

REFERENCES

Boissier, J.R., Tardy. J., Diverres, J.C., 1960. A new simple method to explore action: the test of the chimney. *Med.Exp*., 3, 81.

Bonilla, E., Medina-Leendertz, S., Diaz, S. (2002). Life Span and stress resistance of Drosophila Melanogaster by long-term supplementation with melatonin. *Exp Gerontol*;37 (5):629-638.

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

Dragicevic N., Copes N., O'Neal-Moffitt G., Jin J.B., Mamcarz M., Tan J., Cao C., Oliese J.M., Arendash G.W., Bradshaw P.C.(2011). Melatonin treatment restores mitochondrial function in Alzheimer's mice: a mitochondrial protective role of melatonin membrane receptor signaling. *J Pineal Research*; 51 (1): 75-86.

Espejo, E. F. (1997). Effects of weekly or daily exposure to the elevated plus-maze in male mice. *Behavioural Brain Research*, 87: 233-238.

Gitto E., D'Angelo G., Romeo C., Aversa S., Salpietro C., Reiter R.J., (2012). The Child. *A Journal of Pediatrics* Vol.1; No. 1/1:1.

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

Karasek M. (2007). Does Melatonin play a role in Ageing process? *Journal of Physiology and Pharmacology* 2007, 58, Suppl6, 105-113

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

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

Pierpaoli W., Regelson W., (1994). Pineal Control of ageing: Effect of melatonin and pineal grafting on aging mice. *Proc Natl Acad Sci USA* 91:787-791.

Waldhauser F., Weiszenbacher G., Tatzer E. (1988). Alterations in nocturnal serum melatonin levels in humans with growth and ageing. *J Clin Endo Metab*.66(3): 648-652.

Wang, X., Sirianni, A., Pei, Z., Cormier, K., Smith, K., Jiang, J., Zhou, S., Wang, H., Zhao, R., Yano, H., Kim, J.E., Li, W, Kristal, B.S., Ferrante, R.J., Friedlander, R.M. (2011). The melatonin MT1 receptor axis modulates mutant Huntingtin-mediated toxicity. *J. Neurosci.* 31, 14496–14507.