

Assessing the Effect of Cigarette Smoke Exposure on Spatial Memory in Wistar Rats

M.I. Badamasi and U.G. Esomonu

Department of Human Anatomy, Bayero University Kano, Kano State, Nigeria

Abstract: This study aims at correlating the relationship that could exist between chronic cigarette smoke exposures on the Spatial Memory of Wistar rats in the context of repetitive Morris water maze training supposedly sufficient to attain consolidation of memory. Twenty four (24) male Wistar rats were used for the experiment. The rats were randomly placed into 3 groups: Groups A and B was the treatment group while group C served as the control group. The groups were subjected to 10 episodes of Morris water maze training for 2 weeks with a probe trail test at the end. Group A rats were exposed to cigarette smoke maintained at concentration of carbon monoxide of 100-200 ppm for 10 min while Group B rats were exposed to 300-400 ppm for 10 min. All exposures were for 3 days in a week for 13 weeks. Another probe trail test was carried out at the end of the 13 weeks cigarette smoke exposure period. Probe trail time recording before and after rats exposure depicts the time the Rats spends in the quadrant used to house the escape platform was removed within 60 sec. Nicotine test strip was used to test the urine samples of the Wistar rats after the exposure sessions to verify that cigarette smoke was actually inhaled. The result showed that the Wistar rat's performance in the Probe trail test of Morris water maze showed no statistically significant difference within each group. These finding suggested that whatever effect cigarette smoke may have on the information stored within the 10 training episodes spread over 2 weeks, it didn't alter the findings of similar result following 13 weeks of cigarette smoke exposure with intermittent Morris water maze training.

Keywords: Carbon monoxide, cigarette, nicotine, spatial memory

INTRODUCTION

Some animals due to the peculiarities of their life circle and their natural habitat, do migrate to distant lands just to avoid the harsh winter weather of their present habitat, some also migrate in search of breeding grounds. The fact that these animals can move to and fro these environments proves that they know their way around, which means that most of them have to memorise the path they took and these information according to Mackintosh (2002), are remembered and stored for days or months. The ability of rats to remember escape routes have also been accessed by Mackintosh (2002), within the laboratory. In his experiment, rats were trained to run through mazes to a single goal box and to swim to submerged platform in a circular pool so as to avoid drowning. These abilities fall under spatial memories and they are said to be long term, explicit and episodic memories (Conrad, 1964).

Authors like Toumane and Durkin (1993) in their study on time gradient for post-test vulnerability to scopolamine-induced amnesia following the initial acquisition session of a spatial reference memory task in mice revealed that any event which substantially changes the function of the hippocampus, be it transient, temporarily or permanently will cause amnesia in humans and rats.

Ali *et al.* (2010) studied the effects of iron supplementation on short-term and long-term memory

in adult male wistar rats. They treated the rats with different doses of iron (0, 10, 20, 30, 50, 70, 100, 120, 150 mg/kg) by gavages for 3 consecutive days after which they ascertained the effects of iron supplementation on passive avoidance memory using shuttle box. Their results showed that iron supplementation (20 and 30 mg/kg) significantly improved short term memory ($p < 0.01$, $p < 0.001$) and also, 30 mg/kg significantly increased long term memory ($p < 0.001$) but on the contrary 100 mg/kg impaired ($p < 0.05$) short term memory.

Cigarette smoke has numerous drug constituents of which Carbon monoxide and Nicotine are among the main constituents (Rudnick, 1998). It is a well known fact that smoking cigarette does affect the health of the individual. Cigarette smoking have been revealed to cause structural changes to certain regions of the brain especially the apoptotic bodies in hippocampus (Eser *et al.*, 2007), in the temporal cortex, it has been reported that it could causes reduction in the total number of cells (expressed as reduced DNA) with hypertrophy of the remaining cells (increased total protein/DNA ratio) (Roy *et al.*, 2005).

The association of erythrocytes and leukocytes count and haemoglobin level with cigarette smoking was studied by Zafar *et al.* (2003). They revealed that the erythrocytes and leukocytes counts in smokers was significantly higher when compared to the non-smokers

while the difference in haemoglobin level was significantly lower and when they evaluated the number of cigarettes smoked per day and length of smoking in relation to the blood count, negative correlation was noted with erythrocytes count and haemoglobin level while positive correlation was noted with leukocytes. They therefore concluded that the number of cigarettes smoked per day and length of smoking period do adversely affects blood parameters and may therefore lead to various physiological disorders. They are of the opinion that social habits like smoking should be considered when interpreting the blood haematology values.

Despite the dangers posed by cigarette smoking, the prevalence of the habit has been increasing in Nigeria. There is a dearth of data regarding the effect of cigarette smoke on spatial memory in mammals such as Wistar rats. It is hoped that the present study will shed more light on the effect of cigarette smoke (not individual constituents like nicotine and carbon monoxide) on the spatial memory of Wistar rats.

MATERIALS AND METHODS

Twenty Four wistar male rats were obtained from the animal house of the Biological sciences Department of Bayero University Kano, Nigeria and transported to the animal house in the Department of human anatomy, Ahmadu Bello University Zaria, Kaduna State Nigeria on the 15th day of January 2012. The male Wistar rats were of same average age at the commencement of the experiment (3 months) with weight range of 170-250 g. The rats were randomly divided into three (3) equal groups: Groups A, B and C. Groups A and B was the treatment group while group C served as the control group. The rats were feed with water and chow obtained from Sanders feed Jos, Nigeria ad libitum. The rats were allowed 12 hourly circles of day and light. After the acclimatization period, the rats were subjected to 10 episodes of Morris water maze training for 2 weeks with a probe trail test at the end of the training designated as Early Probe Time (EPT). Morris water maze task involves, training the rats to swim in a tank of 2 m wide and 60 cm deep while they search and locate a hidden platform. The time it takes a rat to locate the platform was noted as Reference time. The tank and the escape platform were painted black to obviate any clue to aid the rats in seeking the platform, sited in the North eastern quadrant (Fig. 1) other than via spatial memory ability. Once the rat was dropped into the Morris water maze, it will swim in the tank searching for an escape platform. The time it spends



Fig. 1: Wistar rats performing water maze task



Fig. 2: Plastic exposure chamber with wistar rats in situ

in locating the quadrant that house the escape platform is called Probe trail time-a measure of spatial memory.

Then rats of group A were exposed to cigarette smoke maintained at concentration of carbon monoxide of 100-200 ppm for 10 min while Group B rats were maintained at concentration of carbon monoxide of 300-400 ppm for 10 min also (Fig. 2). Group C did not receive any cigarette smoke exposure. All exposures for the treatment Groups were for 3 days/week for 13 weeks. Another probe trail test was carried out at the end of the 13th week cigarette smoke exposure period this was designated as Late Probe Time (LPT).

Probe trail time recording before and after rats exposure depicts the time the Rats spends in the quadrant that use to house the escape platform which was removed within 60 sec.

Nicotine test strip that detects the presence of cotinine (≤ 200 mg) a metabolite of nicotine in the urine was used to test the urine samples of the Wistar rats after the exposure sessions to verify that cigarette smoke was actually inhaled. Data was analyzed using SPSS version 16 computer software program.

Table 1: Showing the EPT and LPT for group A, B and C rats

| Group | EPT (Sec) | LPT (Sec) |
|-------|------------|------------|
| A | 35.50±0.89 | 37.75±0.11 |
| B | 29.00±0.45 | 32.50±0.91 |
| C | 27.90±0.45 | 28.00±0.45 |

RESULTS

The result involves the comparison for statistically significant difference between the Early Probe Time (EPT) records with the Late Probe Time (LPT). The findings show that there isn't any significant difference between the EPT and LPT records before and after thirteen weeks of the cigarette smoke exposure regimen.

Group A and B rats recorded a mean Late Probe Time (LPT) of 37.75±0.11s and 32.50±0.91s respectively, which was different from their early probe time with the LPT been higher in magnitude, however, the difference was not statistically significantly. The difference between the LPT and EPT was also not significant for the control group C (Table 1)

DISCUSSION

The present study revealed that there were no significant differences between the probe time records before and after 13 weeks of the cigarette smoke exposure regimen. These findings on one hand buttress the findings regarding hippocampal dependent memory consolidation that suggested that the organization of the memory trace is the important factor in memory consolidation (Squire *et al.*, 1984). Specifically, over the course of time and through a process of competition among memory traces for mnemonic units in the cerebral cortex, the hippocampus binds together the distributed sites in the neocortex that together represent the memory of a whole event (Squire, 1992). However on the other hand, cigarette smoke have been implicated in structural changes to different brain regions like the apoptotic bodies in hippocampus (Eser *et al.*, 2007), reduction in the total number of cells (reduced DNA) with hypertrophy of the remaining cells (increased total protein/DNA ratio) in the temporal cortex (Roy *et al.*, 2005). Superimposed on these patterns, is an overall increase in the membrane/total protein ratio, which is compatible either with smaller cells (higher surface-to-volume ratio) or with increased neuritic sprouting (Slotkin *et al.*, 2006). These results are consistent with studies which postulated that the hippocampus can be dispensable once memories have been sufficiently consolidated in the cortex (Anagnostaras *et al.*, 1999). It has also been reported

that even in the presence of potentially toxic onslaught to the hippocampal apparatus, consolidation of the memory/ability will not be affected as depicted by the histological findings of Eser *et al.* (2007).

Tehranipour and Ebrahimpour (2009) similarly evaluated the effect of aquatic extract of *cannabis sativa* seed on spatial memory consolidation in rats. Similar method using Morris water maze was used to test of the spatial memory. Hemp extract with doses 5, 100, 150 and 210 mg/kg was Injected in the Peritoneal (IP), respectively in the experimental groups while the control group was injected with sterile distill water. Their findings show that, Injected Cannabinoid components can be effective on memory and learning processes especially when consumed in low and medium doses (5, 100, 150 mg/kg) but in high doses it may result to spatial memory weakness unlike cigarette smoking which has no effect on spatial memory as revealed in the present study. Tehranipour and Kafae (2010) further studied the effects of exposure to Extremely Low-Frequency Magnetic Field (ELFMF) of 4 µt intensity on spatial memory and learning in mice and similar effect on the spatial memory as revealed by the Cannabinoid components was also noted as they found out that the exposure to these ELFMF produces a significant increase in the spatial memory of mice just like the *Cannabis sativa*. These reports are contrary to what was revealed with cigarette smoke in the present study.

CONCLUSION

In this study, cigarette smoke exposure maintained at 100-200 and 300-400 ppm, respectively of Carbon Monoxide seemingly has no effect on the spatial memory ability of male Wistar rats in the context of Morris water maze.

REFERENCES

- Anagnostaras, S.G., M. Stephen and M.S. Fanselow, 1999. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: Within-subjects examination. *J. Neurosci.*, 19: 1106-1114.
- Ali, M.A., K.S. Maryam and P.G. Ali, 2010. Dose-dependent effects of iron supplementation on short-term and long-term memory in adult male wistar rats. *J. Biol. Sci.*, 10: 648-652.
- Conrad, R., 1964. Acoustic confusions in immediate memory. *Br. J. Psychol.*, 55: 75-84.

- Eser, O., M. Cosar, O. Sahin, H. Mollaoglu, M. Sezer, M. Yaman and A. Songur, 2007. The neuroprotective effects of Caffeic Acid Phenethyl Ester (CAPE) in the hippocampal formation of cigarette smoke exposed rabbits. *Pathology*, 39: 433-437.
- Mackintosh, N.J., 2002. Do not ask whether they have a cognitive map, but how they find their way about. *Psicologica*, 23: 165-185.
- Roy, T.S., V. Sharma, F.J. Seidler and T.A. Slotkin, 2005. Quantitative morphological assessment reveals neuronal and glial deficits in hippocampus after a brief subtoxic exposure to chlorpyrifos in neonatal rats. *Develop. Brain Res.*, 155: 71-80.
- Rudnick, J., 1998. *Encyclopedia of Occupational Health and Safety*. 4th Edn., International Labour Office, Geneva, pp: 15-37.
- Slotkin T.A., F.J. Seidler, D. Qiao, J.E. Aldridge and C.A. Tate, 2006. Effects of prenatal nicotine exposure on primate brain development and attempted amelioration with supplemental choline or vitamin C: Neurotransmitter receptors, cell signaling and cell development biomarkers in fetal brain regions of rhesus monkeys. *Neuropsychopharmacol.*, 30: 129-144.
- Squire, L.R., 1992. Memory and the hippocampus: A synthesis from findings with rats, monkeys and humans. *Psychol. Rev.*, 99: 195-231.
- Squire, L.R., N.J. Cohen and L. Nadel, 1984. The Medial Temporal Region and Memory Consolidation: A New Hypothesis. In: Weingartner, H. and E.S. Parker (Eds.), *Memory Consolidation: Psychobiology of Cognition*, Erlbaum, Hillsdale, NJ, USA, pp: 272.
- Tehranipour, M. and S. Ebrahimpour, 2009. Evaluating the effect of aquatic extract of *Cannabis sativa* seed on spatial memory consolidation in rats. *J. Biol. Sci.*, 9: 884-888.
- Tehranipour, M. and M. Kafaee, 2010. Effects of exposure to extremely low-frequency magnetic field of 4 μ T Intensity on spatial memory and learning in mice. *J. Biol. Sci.*, 10: 67-70.
- Toumane, A. and T.P. Durkin, 1993. Time gradient for post-test vulnerability to scopolamine-induced amnesia following the initial acquisition session of a spatial reference memory task in mice. *Behav. Neural. Biol.*, 60: 139-151.
- Zafar, I., K.N. Mohammad, M. Nisar, M. Rashida, Assadullah, B. Shumaila and S.A. Mohammad, 2003. Effect of cigarette smoking on erythrocytes, leukocytes and haemoglobin. *J. Med. Sci.*, 3: 245-250.