

FOUR-ARM RADIAL OPEN MAZE (FAROM) AS A TOOL FOR ASSESSING THE EFFECT OF ATROPINE IN SPATIAL MEMORY OF THE RATS

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Summary : Place learning behaviour for working (short term) memory and reference (long term) memory is studied with the Four-arm radial open maze (FAROM) in 18 rats divided equally in three groups. In group I, 0.5 mg of atropine was injected intra-peritoneally 30 minutes before the trial. In group II, saline and in group III Glycopyrrolate were injected instead. Twenty three hungry animals were tested on each day in the maze to search for food kept in one of the eight cul-de-sacs of maze. The latency i.e. the time to reach the goal cul-de-sacs, as well as the error score i. e. the number of entries in the non-goal cul-de-sacs were counted during six consecutive trials, per day. Each trial duration was 5 minutes or the time taken by the animal to search the goal compartment whichever was less. The inter-trials period was 10 min and the work was carried out for a period of 3 weeks. The results show that atropine does block effectively both the memory faculties i. e. working and reference memory and that level of memory deficit induced by atropine is related to the rate of drug uptake by the central cholinergic receptors.

Key words : atropine four-arm radial open maze (FAROM) working memory (short-term)
reference memory (long-term) glycopyrrolate

INTRODUCTION

It is known that high dosages of atropine in humans have central toxic effects manifested by restlessness, disorientation (in time and space) hallucinations etc. In animals, atropine is known to antagonise the action of cholinergic neurons when it acts directly in the brain areas (1).

It is generally held that acetylcholine neurotransmitter system is involved in the learning and memory (2, 3, 4, 5, 6, 7, 8). Many workers have shown atropine when injected intraperitoneally to act at muscarinic cholinergic receptors including neural substrates concerned with memory function as a

result of which these animals show learning deficit (9, 10, 11). The work done with a variety of lesions in so called memory processing areas or by means of blockade of function, with drugs applied directly to the concerned areas, induced maze learning deficits in rodents (12, 13, 14, 6, 15, 16). In some of these cases, the memory deficit was related to short-term memory i. e. working memory of the rodent (17, 18). In other cases the deficit was related to long-term memory i. e. reference memory, also known as spatial mapping of the rodents; however these animals showed gradual recovery of reference memory (14, 19). We do not know whether the central cholinergic system which is believed to be involved in the learning and memory is concerned with long-term memory or short-term

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memory or both. The discrepancies reported in the literature may be due to the fact that the study of atropine effect was assessed by instillation of the drug at one area at a time and not based on the blockage effect of atropine on all the concerned areas; therefore, when one area was functionally blocked, other areas could compensate the memory function. Secondly, all the memory processing areas may not be exclusively cholinergic (20, 21). Thirdly the tools used for assessing spatial memory function might not have been adequate to study both the components of spatial memory viz. spatial mapping (reference memory) and working memory. In fact, O'Keefe and Nadel (22) advocate the use of open mazes to assess the place learning ability of rodents instead of closed mazes used so far in the past which distort the results of the maze learning behaviour of rodents.

The radial-arm maze has been increasingly used to examine the neural systems involving the spatial memory of rodents and to study the influence of drugs on process of memory and learning (23, 24, 25).

The aim of the present work is to study the overall effect of the blockade of all the place learning areas with atropine injected intraperitoneally in the rats and assess short-term as well as long-term spatial memory deficits, if any, tested with the help of four-arm radial open maze, indigenously prepared.

MATERIALS AND METHODS

Animals: Eighteen adult albino rats were divided in three groups. In Group I (n=6), atropine was injected intraperitoneally before the trials. In Group II (n=6), saline was injected instead, and in Group III (n=6) Glycopyrrolate was injected intraperitoneally, a drug that does not have central effect but prolonged peripheral anticholinergic action and is twice as potent as atropine. The animals were

housed individually in wire meshed plastic cages. Water *ad libitum* was provided in cage, but the animals were deprived of food, to increase the hunger drive for procurement of food. The food was made available to the animals in the goal compartment during six trial periods. The animal was allowed to eat the food after entering in goal compartment only for a period of 30 seconds. Food was provided to the animal subsequently in the home cage for a period of one hour after the end of last trial for the day. This 23 hour restriction schedule was adequate to keep the animal hungry but not to the extent of reducing its body weight more than 20% of initial weight.

Apparatus: The place learning behaviour of animals was studied with the help of four-arm radial open maze, indigenously prepared, detailed description of which is published elsewhere (19). To describe in brief, the maze has four radial arms; each arm projecting from the centre has two cul-de-sacs at the end. In all these 8 cul-de-sacs, trays were kept of which only one tray-goal tray contained powdered food. A small metallic start platform was kept at the centre of the maze. The maze was cleaned everyday before the trials and also between the trials.

Behavioural testing: During first two days the animals were allowed to get used to the maze and locate food in the goal tray situated in the frontal cul-de-sac of right arm. From third day onwards, saline/drugs were injected intraperitoneally at the beginning of trials for each day. In Group I, 0.5 mg of atropine in 1 ml solution was injected whereas in Group II, 1 ml of saline was injected and in group III 0.5 mg of glycopyrrolate in 1 ml was injected intraperitoneally. Each animal was tested 30 minutes after injection of the drug. The experimental as well as control rats had six trials per day, the duration of each trial was 5 minutes or the time taken by the animals to reach the goal compartment, whichever was less. The inter-trial period was of 10 minutes.

The criteria of assessment of maze learning ability of rat by accounting the latency and the error scores is described in our previous work in closed-maze (14). The *latency* was accounted by the time (in seconds) taken by the animal to reach from start platform to goal tray. The *error score* was accounted by the number of entries of the animal in the non-goal cul-de-sacs. Both these parameters were visually monitored by two observers. The experiments were carried out at fixed period of the day and under quiet environment. The work was carried out for a period of 3 weeks.

RESULTS

The animals were tested in the maze half an hour after intraperitoneal injection of drugs. Both, experimental and control rats demonstrated good appetite, maintained self grooming behaviour, and

engaged in normal exploratory behaviour in maze. It was noted that atropinised rats appeared more active in the maze as compared to other groups.

The statistical data of performance of the animals of all the 3 groups is indicated in the Table I. The error Score as well as latency which are the 2 parameters studied for assessment of the performance, show that there was no statistical difference between Group II (Saline) and Group III (glycopyrrolate) animals. The results of the Group I (atropine) were highly significant as compared to saline animals. This indicates that the impairment of maze learning performance shown by the Group I animals was due to only the central effects of atropine and not due to the peripheral cholinergic blocking effect of atropine.

TABLE I : Statistical data of Error score and Latency of Group I (atropine) Group II (saline) and Group III (glycopyrrolate) animals.

| Performance | Group | Error Score | | P Values | Latency | | P value |
|---|-------------|-------------|--------|-------------|---------|--------|------------|
| | | Mean | SE | | Mean | SE | |
| Performance of all the trials for 21 days | I | 2.24 | 0.1534 | P 0.05** | 22.33 | 11.824 | P<0.001*** |
| | II | 3.3238 | 0.819 | | 16.238 | 2.030 | |
| Performance of trials for each day | I | 2.24 | 0.16 | P 0.0001*** | 28.3 | 3.600 | P<0.01** |
| | II | 0.34 | 0.520 | | 6.1 | 0.2155 | |
| Performance of trials for each day | II | 0.34 | 0.520 | P .9* | 6.1 | 0.2155 | P<1* |
| | III | 0.36 | 0.410 | | 7.98 | 0.89 | |
| Performance of last and First trial 21 days | Last trial | 2.62 | 0.165 | P 0.001*** | 31.6 | 2.158 | P<0.001*** |
| | First trial | 1.4 | 0.086 | | 11.28 | 0.42 | |

* Not Significant
 ** Significant
 *** Highly Significant

The day-to-day maze learning curves of saline and atropinized rats are displayed in the Fig. 1. The average errors committed by animals on each day as well as the time spent to find the goal compartment, is seen to be significantly increased in atropinised animals as compared to control saline animals. The slope of the curve extending for a period of 21 days is suggestive of partial maze learning by the animal but the spatial learning deficit shown by these animals was never reduced to the controlled saline level as long as daily instillation of atropine was continued. The third week's performance of the animal is seen to be slightly improved as far as latency was concerned nevertheless, the animals were unable to decrease the latency to the level of saline animals. The day-to-day performance of animals in the maze can be accounted for long-term memory which is shown to be impaired under present experimental set-up.

Figure 2 illustrates the learning curve which is plotted on the basis of six consecutive trials performed on each day. It is to be noted that the first trial is spaced 23 hours after the last trial of previous days whereas the rest of the five trials took place at an interval of 10 min. Therefore, one can consider the performance of animals at first trial as related to long-term memory whereas the performance of subsequent trials repeated at 10 min interval may indicate to be related to both, long-term as well as short-term memory. The average error and average latency computed for 21 days at each trial was shown to be progressively impaired (AVG error 2.24 and AVG latency 28.3s) as compared to saline animals (AVG error 0.34 AVG latency 6.1s). This is because the atropinised animals were unable to reduce the number of errors made in the maze and also were unable to reduce the time to reach goal tray despite of appearing generally more active inside the maze.

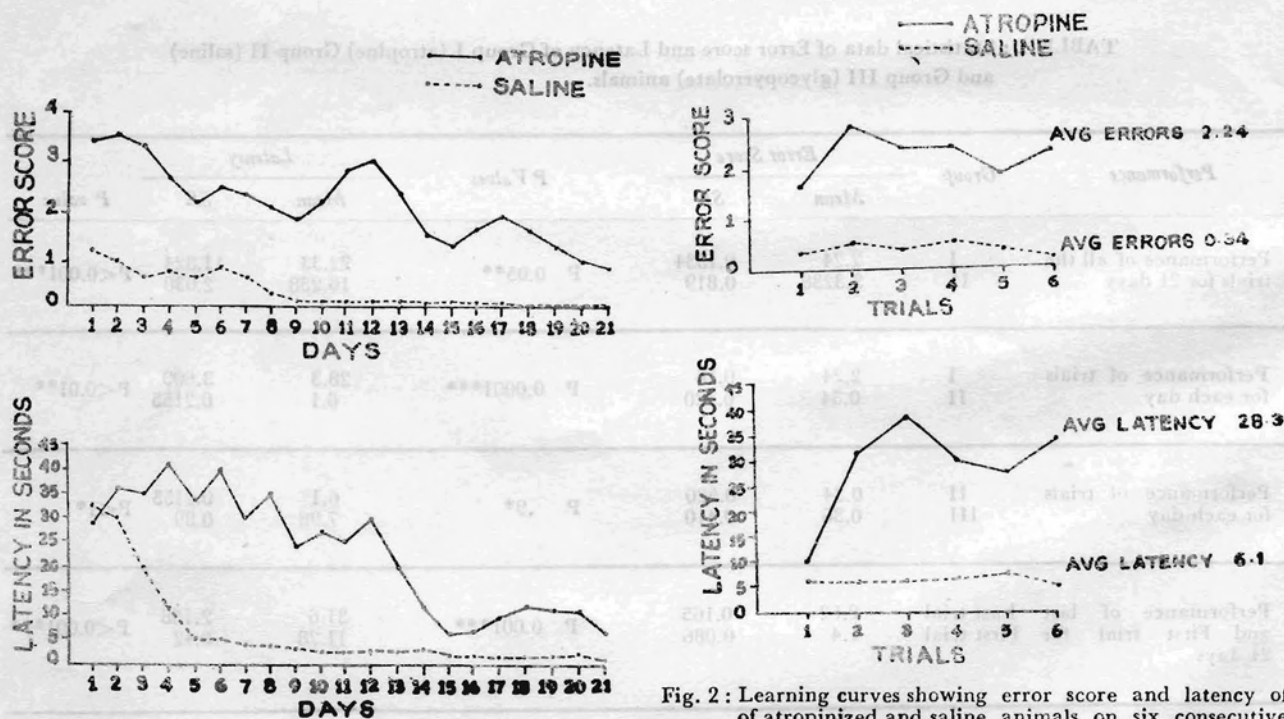


Fig. 1: Day to day error score and latency of atropinized and saline animals for a successive period of 21 days. The average errors as well as the latency is seen to be significantly increased in atropinized animals as compared to the control saline animals.

Fig. 2: Learning curves showing error score and latency of atropinized and saline animals on six consecutive trials of the day. Each point of the error curve is the mean of the errors committed by the animals during 21 days at that trial. Each point of the latency curve is the mean of the latencies accounted during 21 days at that trial. The values indicated in the diagram are the average values of all the six trials.

The learning curve of the 1st trial when plotted against the learning curve of the last trial for both the paradigms viz. latency and error score revealed that the last trial curve remained always at higher values indicating that the maze learning ability of the animal gradually deteriorated 60 min after the 1st trial i.e. 90 min after administration of atropine. (Fig. 3). This is likely to be due to the progressive blockade of central cholinergic receptors subject to the rate of absorption of the drug from the peritoneum or rate of drug uptake at the level of receptors.

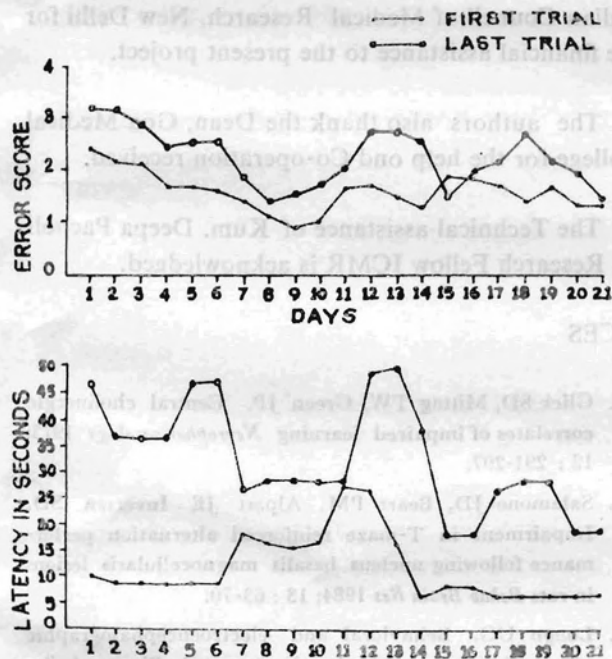


Fig. 3: Learning curves of the first trial plotted against learning curves of the last trial in atropinized animals. The error score curve as well as the latency curve of the last trial remain above the first trial values throughout the experimental period of 21 days.

DISCUSSION

The primary purpose of this work was to find out whether atropine injected intraperitoneally blocked short-term memory or long-term memory or both in the rodents. The learning behaviour was studied with the help of four-arm open radial maze which allowed to study both the modalities of the

spatial memory. The results of this work have clearly demonstrated that atropine does block effectively both the faculties viz. short-term as well as long-term memory.

The experimental work on animals and the clinical evidence is in favour of suggesting that short-term memory which lasts for few seconds to a few minutes depends on the integrity of neocortex while long-term memory that lasts for hours, days and even years depends upon the integrity of both, neocortex and hippocampus (26). It is speculated that hippocampus plays a leading role in transfer of short-term memory into long-term memory and that the retrieval of past memories depends primarily on hippocampus (23).

The present work leads to confirm the earlier reports that the neural system concerned with memory processing situated in the neo-cortex as well as paleocortex (hippocampus) are basically cholinergic. And therefore it is not surprising that both the memory modalities were impaired by atropine. This conclusion should not ordinarily rule out the existence of chemical mediators other than acetylcholine, that may also have a role to play in the memory processing (21). In fact, the atropine injected intraperitoneally in experimental animals could not totally suppress the maze learning ability of the rats (long-term memory) as revealed by gradual decline of the maze learning curve studies for a period of 3 weeks.

It is known that atropine when injected into the body has its peak action within 30 min to one hour, its half-life is 2.5 hours and it is mostly eliminated within 12 hours; however, its action varies from tissue to tissue depending on the rate of uptake and rate of elimination from tissues (1). In the present work the animals were tested 30 min after intraperitoneal injection of atropine. It is seen from the results that the maze learning deficit shown by the animal at the first trial was the least as compared

to the deficit of the maze learning shown during the rest of the trials taken at 10 min interval. This is likely due to progressive blocking of cholinergic receptors on account of gradual uptake of atropine by the memory processing areas and so one can say that memory deficit induced by atropine is related to the rate of drug uptake by the central cholinergic receptors.

Alike the reports on the drugs that interfere with learning and memory there are also reports on the drugs that enhance central cholinergic system by blocking the breakdown of acetylcholine or through excitation of cholinergic receptors that may facilitate learning and memory (15). It is a well accepted fact that yogic meditation contributes to the improvement of body and mind function by achieving dominant control over cholinergic system. This leads to conservation of body energy and improvement of mental faculties. Memory and learning are the indices

which are linked with all the intellectual faculties and as such it is likely that the practice of yogic meditation have direct effect on cholinergic system controls memory and learning process. Study of all the means that may reinforce learning and memory by way of acting selectively on central cholinergic system should be a pointer for the future investigations in this field of research.

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REFERENCES

- Gilman AC, Goodman LS, Rall JW, Murad F. Atropine, Schopolamine and related belladone alkaloids. In : *Pharmacological basis of therapeutics*. *McMillan Publishing company*. Seventh edition, 1985; 133
- Bartus RT, Dean BL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction *Science* 1982; 217 : 408-417.
- Buresova O, Bures J. Radial maze as a tool for assessing the effect of drugs on the working memory of rats *Psychopharmacology (Berlin)* 1982; 77 : 268-271.
- Davis KL, Mohs RC, Tinklenberg, Plafferbaum A, Hollister LE, Kopell BS. Physostigmine, improvement of long-term memory processes in normal humans. *Science* 1969; 20 : 272-274.
- Drachman DA, Sakakian BJ. Memory, aging and pharmacosystems In : *The psychobiology of Aging : Problems and perspectives*, edited by D. G. Stern. Amsterdam : *Elsevier North-Holland* 1980; 348-368.
- Friedman E, Luer B, Kuster J. Loss of cholinergic neurone in the rat neocortex produces deficits in passive avoidance learning : *Pharmacol Biochem Behav* 1983; 19 : 19 : 309-312.
- Glick SD, Mittag TW, Green JP. Central cholinergic correlates of impaired learning *Neuropharmacology* 1973; 12 : 291-297.
- Salamone JD, Beart PM, Alpert JE Inversen SD. Impairment in T-maze reinforced alternation performance following nucleus basalis magnocellularis lesions in rats *Behav Brain Res* 1984; 13 : 63-70.
- Longo UG. Behavioral and electroencephalographic effects of atropine and related compounds *Pharmacol Rev* 1966 : 18 : 965.
- Montoya CP, Sainsbury RS. The Effects of Entorhinal cortex lesions on type 1 and type 2 Theta *Physiol Behav* 1985; 35 : 121-126.
- Vanderwolf CH, Limbic - diencephalic mechanism of voluntary movement *Psychol Rev* 1971 : 78 : 83-113.
- Caulfield MP, Fortune DH, Robert PM. Stublely JK. Intracerebroventricular hemicholinium 3 (HC-3) impairs learning of a passive avoidance task in mice. *Proceedings of the British Physiological Society* 1981 (September); 16-18.
- Dhume RA, Gogate MG. Significance of spatial and temporal dysfunction exhibited by enhanced running

- activity in hippocampally - lesioned rats *Ind J Physiol Pharmac* 1983; 27(3) : 209-216.
14. Dhume RA, Noronha A, Nagvekar MD, Gogate MG. Effect of lesion in ventral hippocampus of rats on cognitive mapping *Indian J Med Res* 1986; 84 : 398-404.
 15. Hurlburt JB, Lubar JF, Switzer R, Dougherty J, Eisenstadt ML. Basal forebrain infusion of HC-3 in Rats : Maze learning deficits and Neuropathology *Physiol Behav* 1987; 39 (3) : 381-393.
 16. Sutherland RJ, Whishaw IQ, Regehr JC. Cholinergic receptor blockade impairs spatial localization by use of distal cues in the rat *J Comp Physiol Psychol* 96 : 563-573.
 17. Davis HP, Tribuna J, Pulsinelli WA, Valpe BT. Reference and working memory of rats following hippocampal damage induced by transient forebrain ischemia *Physiol Behav* 1986; 37(3) : 387-292.
 18. Morris RGM. An attempt to dissociate "Spatial-Mapping" and "Working-Memory" theories of hippocampal function. In : *Neurobiology of the Hippocampus* ed. by W. Seifert London : Academic Press, 1983.
 19. Dhume RA, Noronha A, Nagvekar MD, Gogate MG. Role of hippocampus in cognitive mapping tested in rats using radial four-arm open maze *Proceedings of second Indo-Soviet symposium on Brain and Psychophysiology of stress at Delhi (India), 1987 January; 22-24.*
 20. Alvarez ED, Guerra FA. Effects of Histamine micro injections into the hippocampus on open-field behavior in Rats *Physiol Behav* 1982; 28(6) : 1035-1040.
 21. Sahgal A. Functions of hippocampal system *Trends in Neurosciences*, 1980; 3(5) : 89-93.
 22. O'Keefe, J, Nadel L. *The hippocampus as cognitive mop* (Oxford University Press) 1978.
 23. McCann DJ, Rabin RA. Winter JC. Use of the radial maze in studies of phencyclidine and other drugs of abuse. *Physiol behav* 1987 ; 40(6) : 805-812.
 24. Olton DS. The radial-arm maze as a tool in behavioral Pharmacology *Physiol Behav* 1987; 793-797.
 25. Walsh TJ, Chrobak JJ. The use of the radial arm maze in neurotoxicology *Physiol Behav* 1987 ; 40(6) : 799-803.
 26. Kolb B. Whishaw IQ. *Fundamentals of Human Neuropsychology.* *San Francisco Freeman.* 1980.

MATERIALS AND METHODS

The subjects belonged to a group of low socio-economic group attending a contraceptive testing centre. The details of selection of subjects for this study are same as in our earlier study (4). The subjects were administered orally a pill containing either 1 mg or 300 µg NET-acetate along with 50 or 50 µg ethinyloestradiol (EE2) respectively, on empty stomach. The tablets were prepared and supplied by IDPL Hyderabad. Blood samples were drawn at 0.5, 1, 2, 4, 8, 12 and 24 hr after administration

INTRODUCTION

The synthetic steroid, norethisterone (NET), is one of the contraceptive progestational steroids often used singly or in combination with other steroids. In our earlier study with NET mini pill (5) we found that plasma elimination half-life of NET was faster in women from low socio-economic group as compared to high socio-economic group. Hence, if there is the possibility that norethisterone may cause increased drug failures, these combination pills are more commonly used, the pharmacokinetics of NET were evaluated in