Effect of acute tritiated water (HTO) exposure on Maze learning in adult Swiss albino mice

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ABSTRACT

Background: Brain is the most important organ as it controls and co- ordinates all the activities of our body. Reports on neuroethological investigations in mice particularly associated with learning and memory after radiation exposure are very scanty. Hence, present investigation is an attempt to examine the effects of low dose tritiated water (HTO) acute exposure on adult Swiss albino mice in the light of behavioral parameters. ***Materials and Methods***: Swiss albino adult male mice were trained in a Hebb William's Maze, model D. When the mice had learned the Maze, they were injected with tritiated water and then the same batch (pre learned mice) again tested for memory retention. ***Results***: Our results clearly show that the adult mice learned the Maze in ten trials. On day ten of the experiment mice were injected with dose 111.0 kBq/gm. bd. wt. tritiated water. Immediately after the exposure, the mice started taking relatively more time to reach the goal which continued till trials 11, 12 and 13 of the experiment. However, after this, a reversal in the learned performance was observed in most of the experimental mice whereby the learned activity returned to a near normal on trials 14 and 15. From 16th trial onwards, the performance of the irradiated mouse was found to be even superior over the prelearned mice though, their pattern did not show the steady state as was evident just prior to HTO injection. ***Conclusion***: The findings from our experiments signify that the possible alterations in behaviour in adult Swiss albino mice after low doses of acute tritiated water exposure cannot be safely ruled out.

Keywords: *Brain, Maze, tritium, tritiated water, learning.*

INTRODUCTION

Tritium is the heaviest and the only radioactive isotope of hydrogen. It decays to the stable isotope $_3$He by emitting low energy electrons. The short range and low energy of β-rays gives rise to almost complete absorption of the radiation energy within the biological matter. While some beta particles can penetrate human skin, tritium's beta particles do not have enough energy to do so. Tritium is widely distributed throughout the man's environment because of its ubiquitous form as tritiated water and its persistence in the environment [(1)]. It is produced naturally in the upper atmospheric region by the interaction of Cosmic rays with Nitrogen and Hydrogen [(2)]. The tritons in th e upper atmosphere are oxidized to tritiated water (HTO) and mix with the hydrosphere generally through the movement of air masses and precipitation. Emission of tritium from the nuclear fuel cycle will increasingly become the dominant source of this nuclide and can become more important than the residue from weapons testing. Along with Lithium, tritium is the only radioactive product present in the breeding reaction in large quantities; it may create problems both from leakage during routine operation as well as in the event of an accident. It requires of

course, most careful containment and recovery systems as has been discussed by Watson [3] and Häfele *et al*. [4].

Though, tritium exposure in the form of HTO has been considered not very toxic, yet the metabolism of tritium in mammals needs a careful evaluation when all forms of tritium have to be taken into account in terms of effects. In fact, the short range of tritium beta particles which label in the cell nucleus is decisive for the biological effects. Tritium incorporated in organic material behaves differently and may be accumulated in biological system.

Incorporation of tritium from tritiated water in mammalian brain has been studied by several investigators [5-7]. Brain is heavily exposed due to more specific activity of organic tritium and its slow turnover. Most importantly, brain is the main organ responsible for controlling higher functions like learning and memory. In order to understand the essentials of the learning process, psychologists have worked mainly with the animals which allow closely controlled experiments in relatively simple situations. Rat and mice are convenient animals for this purpose and one of the favorite instruments for investigating their learning performance is the Maze. Maze is a testing apparatus consisting of passages where only one leads to the goal, while others come to a dead end. Important measure of Maze learning is the time and the number of trials which an animal takes to find the goal without error. Learning experiments in mice post- radiation exposure have been performed long way back by Blair and Arnold [8], Blair [9] Jensh *et al*. [10] and many other workers. Recent investigations [11] on the behavior of adult Swiss albino mice post acute tritiated water (111.0 kBq/gm. bd. wt.) as well as after continuous tritiated drinking water exposure at a dose (11.1 kBq/ml.) report no statistical change in the learning behavior of experimental mice in Maze over the control batches run simultaneously. Present study is an attempt to investigate the effects of acute tritiated water exposure on the learned performance of adult male Swiss albino mice in a Maze.

MATERIALS AND METHODS

Swiss albino mice procured from Cancer Research Centre, Mumbai, India were maintained and bred in an air cooled laboratory. They were fed on balanced food manufactured by Hindustan Liver Limited and water was provided *ad libitum.* Animals were randomly selected from the colony for experimental purpose. Adult male Swiss albino mice categorized into five individual lots as series 1 to series 5 were trained in a Hebb William's Maze, Model D. The mice were left in chamber 'A' of the Maze and allowed to explore the dark Maze to reach chamber 'B' where nice odorous food was kept. Time taken (in seconds) to reach the goal which is chamber B of the maze in our experimentation was noted after every trial. The mice were assumed to have learned the maze when the time taken to reach the goal became almost constant on past several trials. These mice, now designated as pre learned mice were immediately *intramuscularly* injected with 111.0 kBq /gm. body weight HTO and then again tested for memory retention in the Maze. The investigation was performed for a period of 40 days which included 20 trials, the experiment having been performed on alternate days.

Dosimetry

When the dose given has been single, the Initial Dose Rate (IDR) has been calculated. The IDR is defined as the rate at which the energy is imparted to unit mass of tissue. In the present investigation, when the animals were injected with 111.0 kBq/gm. body weight HTO, dose delivered has been calculated to be 0.92 cGy/day.

RESULTS

Our results clearly show that the mice had learned the Maze in Ten trials or 20 days, the experiment having been performed on alternate days. Mice were presumed to have learned the Maze once the time taken to reach the goal (chamber B of the Maze where nice odorous food was kept) became almost static consequently on several of the previous turns of the experiment. They were marked to have taken

almost the same time to reach the goal on trials 8, 9 & 10. Hence, trial ten of the experiment was fixed as the day of injecting the animals with dose 111.0 kBq /gm. bd. wt. tritiated water. Immediately after the exposure, the learned behavior suddenly got disturbed and the mice started taking relatively more time to reach the goal. This means that the exposed mice showed a decline in the learned performance. This trend persisted continuously on trials 11, 12 and 13 of the experiment. However, after this, in most of the experimental mice, a reversal in the performance was observed whereby the learned activity returned to near normal. From figure 1 it can be seen that on 14th and 15th trials, mice started taking almost the same time to reach the goal as they had previously taken on trials 8 to 10. Very contrastingly, from trial 16 onwards, irradiated mouse were found to be superior over the control (prelearned) animals though, their pattern did not show the steady state as was evident just prior to exposure. This tendency lasted till the end (trial20; day 40) of the experiment.

DISCUSSION

In rats and mice exposed to tritiated water for extended periods, the highest relative concentrations were in the brain lipids followed by skin and muscles. Brain being one of the critical organs for tritiated water exposure, it is worthy to establish a co - relation between higher brain functions like learning and memory and radiation exposure.

Maze experiments have played crucial role in understanding the learning behaviour and these experiments are important in understanding the theory of learning. Our results are in good agreement with those of Jones *et al.*[12]) who reported that male rats showed a significant depression in activity for the first four days following exposure to 400 rads X-irradiation which was followed by a recovery. Our results are further in corroboration with the findings of Landaeur *et al.* [13] who exposed female mouse to 300 rads fission neutrons and showed depression in activity which returned to control levels after 5-7 days. Similarly, Maze learned rats whole head irradiated for 2500r were tested on post irradiation days 3, 12, 25, 40, 60 and 80. On the day 3 test, the control rats performed better than the irradiated ones. However, by day 25, there has been a reversal and the irradiated rats were found superior over the controls.

The depression in the learned behavior during the early trials post irradiation may be due to the effects of endotoxin. Irradiated mice exhibit detectable levels of endotoxin in their tissues possibly derived from the endotoxin pool in the intestinal tract. Disruption of intestinal epithelial cell integrity [14] may account for the passage of endotoxin into the blood stream and hence, the resulting deleterious effects observed in the study. The other hypothesized factor governing the decreased performance may be increased hunger motivation and reduced motivation resulting from sickness due to irradiation. Still another explanation for the decrement in activity as observed in our study may be due to prostaglandins. Prostaglandins previously have been reported to depress locomotor activity [15] and to enhance activities of pain at peripheral sites.

However, very interestingly, the initial depression in the learning aptitude post irradiation returns to a normal level after several trials during the next few days which further improves as the experiment continues. This change in learning in the irradiated lot may be correlated to the fact that the changes in the

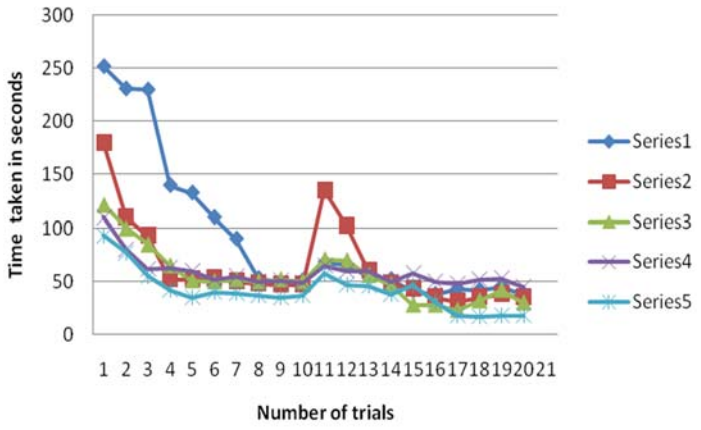

Figure 1. Variation in learning ability in Swiss albino mice in a Hebb William's Maze injected at a dose level 111.0 kBq/gm. bd. wt. HTO.

concentrations of neurotransmitters at the synapse post- HTO exposure may play a significant role in memory retention. Complex learning is largely dependent on the integrity of cerebral cortex in mammals and since, cortex is relatively radio-resistant, it should not be surprising to note that the learned behaviours are not adversely affected post-irradiation. At the cellular level as well, reports on mild radio pathological changes in the brain tissue following tritiated water exposure exist [15] which signify that the possible changes in the behaviour after low doses of ionizing radiations also cannot be safely ruled out. Investigations on alteration in behavioural response to radiations with regard to higher brain functions such as memory and learning in vertebrates are scanty; hence, an extensive research is desirable.

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