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RESEARCH PAPER

THE EFFECTS OF 5-HYDROXYTRYPTOPHAN ON VISUO- SPATIAL LEARNING AND MEMORY IN MICE

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ABSTRACT

The aim of this present study is to investigate the effect of repeated administration of 5-Hydroxytryptophan diet (5-HTP) on learning and memory using CD-1 mice as experimental animals. Twenty male (20) mice were randomly assigned into two groups of ten mice each, namely; Control and 5-Hydroxytryptophan diet (15%w/w) groups. Feeding lasted for 28 days, during which there were daily measurements of food intake, water intake and body weight changes. Thereafter, the Morris water maze task was used to assess their learning and memory abilities through their ability to locate the hidden platform. The results in the Morris water maze test, showed that the swim latency for the 5-Hydroxytryptophan diet group was significantly longer compared to control ($P< 0.05$).The probe trial of the Morris water maze test showed a significantly shorter quadrant duration in the 5-Hydroxytryptophan group (5-HTP) compared to the control($P<0.01$). Therefore, repeated administration of 5-Hydroxytryptophan diet enhances visuo-spatial learning and memory in mice.

Keywords: Morris water maze, 5-Hdroxytryptophan, memory, mice.

INTRODUCTION

5-Hydroxytryptophan is an aromatic amino acid naturally produced from L-tryptophan (LT). It is obtained commercially by extraction from the seeds of the plant *Griffonia simplicifolia*.5-Hydroxytryptophan (5-HTP) is the precursor of the neurotransmitter serotonin(a neurotransmitter that relays signals between brain cells),it has been used clinically for over 30 years**(**Timothy and Birdsall, 1998)and its clinical efficacy anchors on its ability to increase production of serotonin. Some research findings support the use of 5-HTP in the treatment of cerebellar ataxia, headache, depression, psychiatric disorders or aid panic disorder, but studies in people with schizophrenia has shown different results (Bagdy *et al*., 2007) and as an appetite suppressant etc.(Harford *et al*.,2007; Troullas *et al*.,1988.,Bono *et al*.,1984)

It has also been shown that 5-HTP may cause GIT disturbances, mood disturbance, seizure etc. It has also been reported that side effects might result from contaminants in 5-HTP products. However, most of the studies involving the use of 5-HTP were for depression and conducted many years ago. At that time, there was a high level of interest in serotonin hypothesis on depression (Shawk *et al.,* 2002). It is possible that this series of events on depression may have led to the loss of interest **i**n 5-HTP in respect to neurobehaviour. It may be worthwhile, as intended in this study, to find out whether repeated administration of 5-Hydroxytryptophan diet can affect behaviour. This is of particular interest when we

consider the challenges that confront human behaviour and how behavioural disorders still remain a global concern (Messman, 2005).

MATERIALS AND METHODS

Preparation of 5-Hydroxytryptophan diet: 5-Hydroxytryptophan was obtained from May and Baker United Kingdom, and used for the study. The serotonin precursor diet was prepared by mixing one gram (1g) of the 5-Hydroxytryptophan with 99g of the feed. An electric blender was used to blend the mixture to form the serotonin precursor diet.

Determination of lethal dose of 5-Hydroxytrytophan: The acute toxicity of 5HTP was estimated using 25 Swiss white mice weighing between 24 and 25g. The mice were divided into 5 groups consisting of 5 mice per group. Each group of mice for 5HTP was given a different dosage of the extract [1600mg/kg, 800mg/kg, 400mg/kg, 200mg/kg, and 2mls of water for control] all per oral. The number of deaths in each group within 24-72hours was recorded. The LD_{50} was calculated using probit kill of the dose which is the formula or method proposed by Lorke (1983).

Animal Treatment: Twenty (20) Swiss mice weighing between 21g and 30g were randomly assigned into two groups of 10 mice each. Each mouse in a study group was individually housed in a plastic cage with iron gauze bottom grid and a wire screen top. The animal room was adequately ventilated and kept at room temperature and humidity of 22 ± 5^{0}c and 38-68% respectively with 12 hour natural light-dark cycle. They were fed with normal rodent chow and given water freely for 28 days to allow for acclimatization before the commencement of the experiment. The control group received normal rodent chow, while the test group of animals were administered 15g of 5-Hydroxytryptophan diet, daily for a period of 28 days and within this period, their beddings, feed and water intake were hygienically handled and changed daily. Body weights of the animals were also taken every 3 days. Thereafter; the animals were assessed for their learning and memory capabilities.

Assessment of learning and memory: The Morris water maze modified for a mouse was used (Paylor *et al.,* 1996).The water maze was constructed using a circular rubber basin that measures 110cm in diameter and 20cm in depth. The pool was filled to a depth of 14cm with water. The water was made opaque with the addition of liquid milk to ensure camouflage of the white escape platform. The platform was submerged by 1cm of water. The water was left to stay overnight in order to achieve room temperature. The pool was divided into four quadrants: Northwest, Northeast, Southwest and Southeast. Boundaries of these quadrants were marked on the edges of the pool with masking tape and labeled: North, South, West and East. A square solid block (10cm x 10cm) covered with white gloves was used as the escape platform in the maze. The level of water in the pool was adjusted to 1cm above or below the platform. Thus, creating a visible or invisible platform respectively. On the walls of the room were mounted several posters to act as visual cues. During testing, the room was dimly lit with sunlight passing through the window covered with curtains. The performance of the animals in the maze was measured both manually and electronically.

Testing in the Morris Water Maze lasted for eight days. The first three days were acquisition training with the invisible platform. Day 4-6 were reversal training, again with an invisible platform. On the seventh day, a probe trail was conducted with no escape platform. On day eight, four trials were conducted using the visible platform. Sixty (60) seconds were allocated for each mouse to locate the platform in each trial. Mice which were unable to locate the platform were guided to the position of the platform. The timer was stopped when the mice locate the platform within the 60 seconds. The time it took the mice to locate the platform was recorded as the swim latency. After each trial, mice were placed in cages with shredded paper towel beddings to make them try easily and a heating lamp was also provided to prevent animals from developing hypothermia.

STATISTCAL ANALYSIS: Data collected were expressed a Mean ± SEM (standard error of mean), analysis of variance (ANOVA) and the student 't' test were used for analysis. "P" value less than 0.05 and $P<0.001$ was considered statistically significant.

RESULTS

Lethality Study of 5-Hydroxytryptophan: The lethality dose of 5-Hydroxytryptophan following graded doses of 200 to 1600mg/kg was 155.30mg/kg (Fig.1).

Comparison of Swim Latency in the Morris Water Maze Test for Learning and Memory: During the acquisition training, the 5-Hydroxytryptophan group had a significantly shorter (P<0.001) swim latency during the 3 days of training compared to control. This is as shown in (Fig.2).Similarly, the swim latency during the reversal training was also significantly shorter in 5-Hydroxytryptophan group on day 4, 5 and 6 compared to control (P<0.001) (Fig.3).

Comparison of the Quadrant duration in the Morris Water Maze Test for Learning and Memory in the Different Experimental Groups: In the probe trial, the 5-Hydroxytryptophan group showed a significantly longer quadrant duration compared to control (P<0.05) (Fig.4).

Comparison of the Swim Latency during Visible Platform Task of Morris Water Maze Test: During visible platform task, the swim latency of the 5-Hydroxytrytophan group was significantly shorter (P<0.05) compared to control (Fig.5).

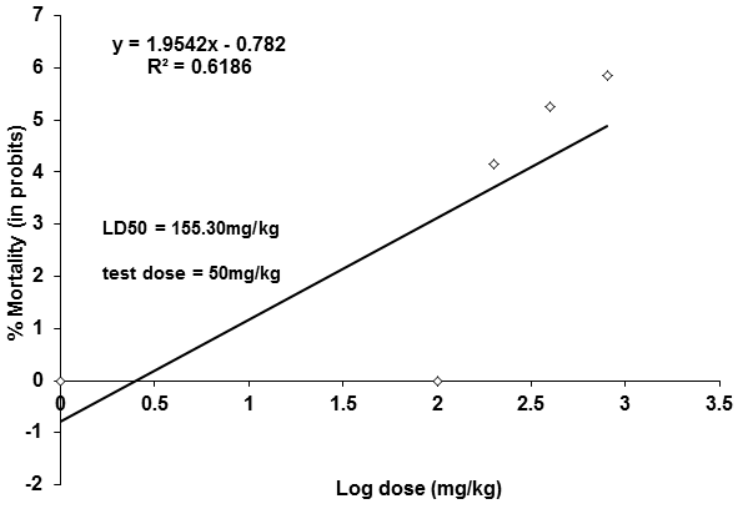

Fig. 1: Lethality studies on the effects of administering graded doses of 5HTP (200 to 1600mg/kg i.p. mice) against the percentage mortalities (converted to probits)

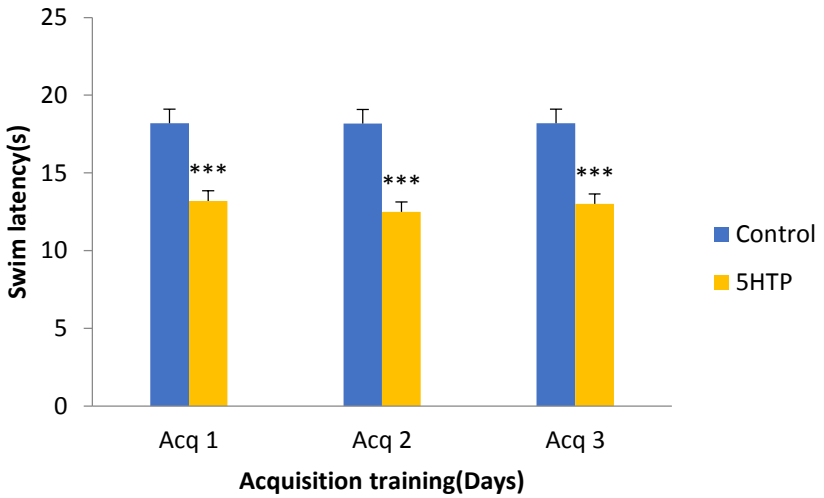

Fig 2: Comparison of swim latency in Morris water maze test during acquisition training of the different experimental groups. Values are expressed as mean, ± SEM, n = 10,*p<0.001 vs. control.

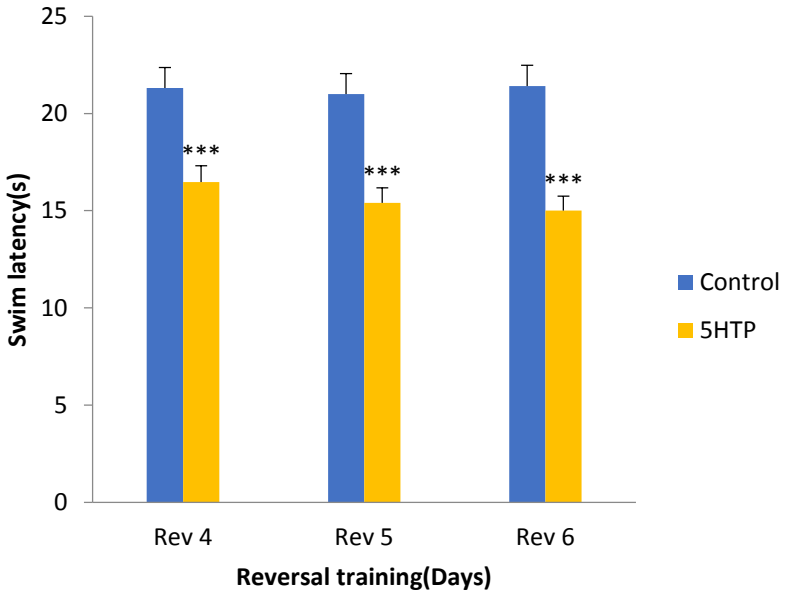

Fig 3: Comparison of swim latency in Morris water maze test during the reversal training of the different experimental groups. Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.001 vs. control.

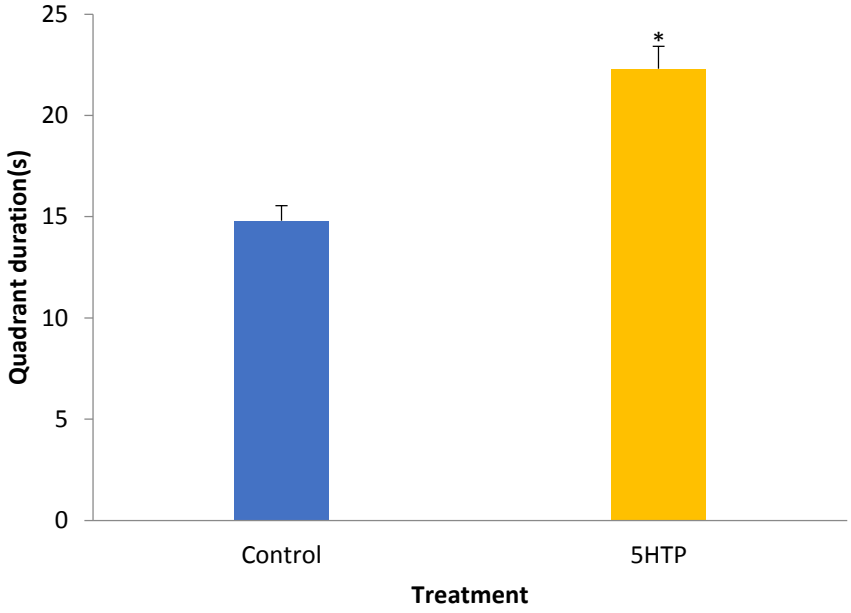

Fig 4: Comparison of quadrant duration in Morris water maze test of the different experimental groups. Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.05 vs. control.

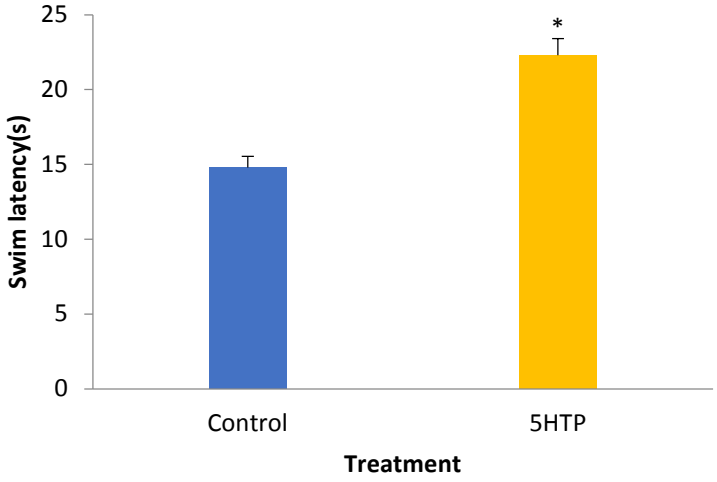

Fig 5: Comparison of the swim latency during visible platform task in the Morris water maze test of the different experimental groups. Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.05 vs. control.

DISCUSSION:

The hidden platform version of Morris water maze is a test of visuo-spatial learning and memory. This process is impaired when the hippocampus is injured (McDonald and White, 1994).The visible (cued) platform uses a unique intra-maze visual cue that is placed at the location of the escape platform whereas the visuo-spatial learning task uses extra-maze cues. The shorter the swim latency, the better the training process. Mice that learn faster were able to identify the spatial location/position of the hidden platform earlier than their counter parts (within a short time).

Following the administration of 5-Hydroxytryptophan diets, swim latencies for the first three days during acquisition training showed that the swim latencies of 5-HTP group was significantly lower compared to the control. During reversal training, the swim latencies for the three days in the mice that consumed 5-HTP diet were all shorter than the control group. This means that this group of mice was able to locate the hidden platform faster and so, learned faster than the control group.

The cued version of the Morris water maze assesses cued learning and visual integrity of the animals tested. In this cueing procedure, the escape platform protrudes above the water surface. Shorter swim latency indicates improved cued learning. From the results, the swim latencies in cued learning were significantly different compared to control. This means that the test group had improved cued learning compared to control (Morris, 1984).

Visuo-spatial memory was assessed during the probe trial in the Morris water maze task. During this trial(60 seconds exploration without hidden platform), it was expected that mice which had a good memory of the spatial location/position of the hidden platform would spend more time exploring the quadrant which had the platform during reversal training, in this case, the retention quadrant was South-East(SE) quadrant. Mice that consumed 5-HTP diet spent significantly more time than the control exploring the retention quadrant. This means that they had better memory than the control group of mice. This is in line with the work by (Aduema, 2016., Walther et al., 2003., Portas *et al.*, 2000).Learning and memory which are complex cognitive functions of the higher nervous centers encompass a variety of subcomponents with many interactions and overlaps (Brem *et al.*, 2013).Memories are stored in the brain by changing the basic sensitivity of synaptic transmission between neurons as a result of previous neural activity. It is likely that the repeated administration of 5-HTP diet enhanced synaptic transmission between neurons by not interfering with the basic sensitivity of the transmission in the hippocampus leading to improved learning and memory of the mice.

In conclusion, long term repeated administration of 5-HTP diet enhances learning and memory in mice.

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AUTHOR(S) CONTRIBUTION.

All authors have contributed one way or the other to the success of this paper and there is no conflict in relation to made technical inputs and interpreted the results, while Ofem, E.O. carried out the statistical analysis.