ORIGINAL ARTICLE

# RELATIONSHIP OF BRAIN TRYPTOPHAN AND SEROTONIN IN IMPROVING COGNITIVE PERFORMANCE IN RATS

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## ABSTRACT

Brain function can be affected by the availability of dietary precursors of neurotransmitters. The diet induced increase in tryptophan (TRP) availability has been shown to increase brain serotonin synthesis and various related behaviors. Evidence shows that TRP and serotonin (5HT; 5 Hydroxytryptamine) play a significant role in memory function. Enhanced brain serotonin activity has been shown to improve cognitive performance in animals and human whereas decreasing brain 5HT levels by acute TRP depletion has been shown to impair cognition. A number of methods have been used for the assessment of memory in animals. In the present study, the radial arm maze and the passive avoidance was used for the assessment of memory in rats following long-term TRP administration. TRP at doses of 50 and 100 mg/kg body weight was orally administered for 6 weeks. The present study shows a significant improvement in memory of rats following both doses of tryptophan. Plasma TRP, brain TRP, 5HT and 5 hydroxy indol acetic acid (5HIAA) levels were increased significantly following administration of TRP. The results of the present study suggest that increase in brain 5HT metabolism following long term TRP administration may be involved in enhancement of memory.

**Keywords:** 5HT, memory, radial arm maze, and passive avoidance.

## INTRODUCTION

TRP is an essential amino acid and its source is dietary only. Increased brain TRP availability has been shown to increase brain 5HT synthesis (Young and Gauthier, 1981; Fernstrom, 1985), as the enzyme TRP hydroxylase is half saturated with its substrate (Hamon *et al*., 1981; Young and Gauthier, 1981). Previously increased TRP load has been shown to increase brain 5HT and 5HIAA levels (Chaouloff *et al*., 1986; Yokogoshi *et al*., 1987). Other studies also show an increase in brain 5HT synthesis following administration of its precursor TRP (Young and Teff, 1989; Feurte *et al*., 2001). 5HT and its precursor TRP are known to have a role in the regulation of various behaviors such as appetite, mood, sleep and cognition (Markus *et al*., 2000; Bell *et al*., 2001). Increased brain serotonin activity is suggested to improve cognitive performance (Levkovitz *et al*., 2003; Laercio *et al*., 2004), whereas a decrease in brain TRP and 5HT levels on the other hand has been shown to impair memory function (Porter *et al*., 2003; Lieben *et al*., 2004). Based on the above consideration, the aim of present study was to investigate the effects of long-term oral administration of TRP on memory function in rats by two different methods i.e. passive avoidance test and 8 arm radial maze test which is known to be an assay of spatial working memory.

## MATERIALS AND METHODS

### *Animals*

Eighteen locally bred albino Wister rats (150-160 g) purchased from the Aga Khan University Animal House were used in the study. All animals were housed individually under a 12 hour light-dark cycle (light on at 6:00 h) and controlled room temperature (22±2°C) with free access to cubes of standard rodent diet and tap water for at least 3-4 days before experimentation so that rats could adapt themselves to the new environment. All experiments were conducted according to a protocol approved by Local Animal Care Committee.

### *Drug administration*

Tryptophan at doses of 50 and 100 mg/kg body weight was used. The experimental protocol was designed to administer tryptophan orally for 6 weeks. Animals were randomly divided into control and two test groups. One test group received 50 mg/kg TRP while other test group received 100 mg/kg body weight TRP daily for 6 weeks. Weighed amount of food was placed in the hopper of all the cages. Body weight and food intake were monitored weekly. Behavioral activities of rats were monitored. Rats were decapitated after 6 weeks between 10:00 and 11:00 h to collect plasma and brain samples. The experiment was performed in a balanced design in such a way that control

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**Fig. 1**: The apparatus used for the Passive avoidance test for memory.


**Fig. 2**: The 8 arm radial maze for assessment of memory used in the present study.

and drug treated rats were killed alternately to avoid the order effect. After decapitation blood was collected in heparinized tubes and centrifuged to get plasma. These plasma samples were then stored below -70°C for estimation of TRP. Brain samples were excised very quickly from the cranial cavity within 30 seconds of the decapitation. Fresh brains were dipped in chilled saline (0.9% w/v) and stored at low temperature (-70°C) until analysis of 5HT, 5HIAA and TRP by HPLC-EC.

***Behavioral tests***

*Passive avoidance test*

Passive avoidance paradigm consists of two compartments as an illuminated 'safe' and a dark 'punishable' one. Both compartments were connected with a door that enable free crossing from one compartment to another. Both compartments had a grid floor. The diameter of rods was 5 mm with 0.5 cm distance between the rods. In the training session, rat was placed in an illuminated box. Once the rats prompted by their instinct stepped its four paws into the dark compartment, rats received 1.5 mA foot shock through the grid floor to its paws. After receiving the foot shock, it immediately came back to illuminated safe compartment. During the test period (24h later), rats were placed in the bright compartment again for a maximum of 5 minutes. The step- through latency that indicates the time elapsed before the mouse entered the dark compartment was recorded in the test session.

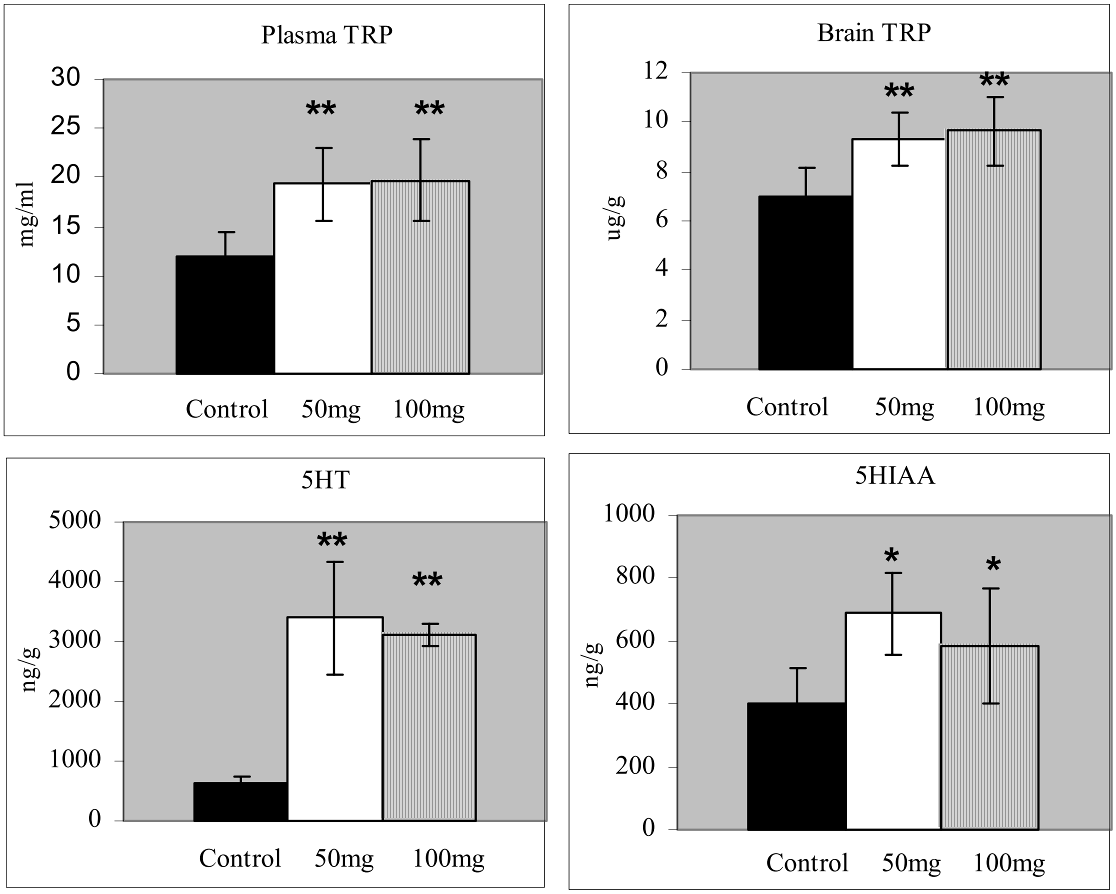


**Fig. 3**: Brain TRP, Plasma TRP, 5HT and 5HIAA in control and TRP-treated (50 mg/kg and 100 mg/kg b.w) rats. Values are mean ± S.D (n=6). Significant difference by Newman Keuls test; $*P<0.05$, $**P<0.01$ vs respective control group.

*Radial maze test*

Testing of cognitive function was performed in an eight arm radial maze. This procedure tests spatial working memory and assesses the integrity of the frontal cortex and hippocampus. The maze utilized in this research study consisted of central platform (32 cm in diameter), which serve as a starting base communicates with 8 arms of equal length (58 cm) and width (12cm) distributed radially and each arm with 38cm high plastic walls. 5 cm from the end of each arm a small plastic receptacle was placed to hold the food out of view from the center of the maze. The apparatus was mounted on a table so that it was 58 cm off the ground. We provided no special means to dispel the effect of smell because in radial maze vision is more important than smell. The maze was placed in laboratory where extramaze cues are there to facilitate learning. To ensure motivated performance, rats were food-restricted to 50% for 1 week before the experiment, but had free access to water in the home cage. The method was essentially the same as described by Neese *et al* (2004) with slight modifications.

RAM testing consisted of 3 phases.

***Habituation***

Rats were given 20 minutes habituation trial on 1$^{st}$ day with free access to all arms, one of which was baited with a hidden food reward. During habituation trial, rat freely visits each arm as many times as it likes. These were neither counted nor recorded.

***Training***

7 arms of the maze were blocked allowing access to only one arm. Food reward was placed at small intervals down the arm in order to entice the rat to the end of the arm. Once the rat reached the end of the arm, the rat was returned to the central platform and the arm was rebaited in order to continue the training procedure. Near the end of 15 min phase, food rewards were only placed at the end of the arm to train the rat to run to end of the arm to receive the reward.

***Testing***

The same arm that was baited with food during the training period was again baited with food during the testing session. The rest of the seven arms were also unblocked and rats had free access to all arms. Each rat was given 5 minutes to

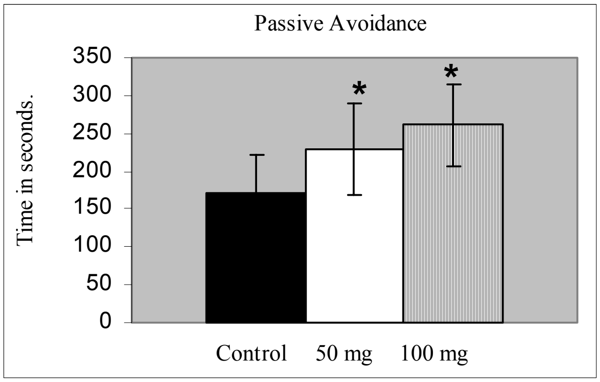


**Fig. 4**: Effect of TRP supplementation (50 mg/kg and 100 mg/kg b.w) on passive avoidance response in rats in the bright and dark compartment. Values are mean ± S.D (n=6). Significant difference by Newman Keuls test; *P<0.05 vs respective control group.

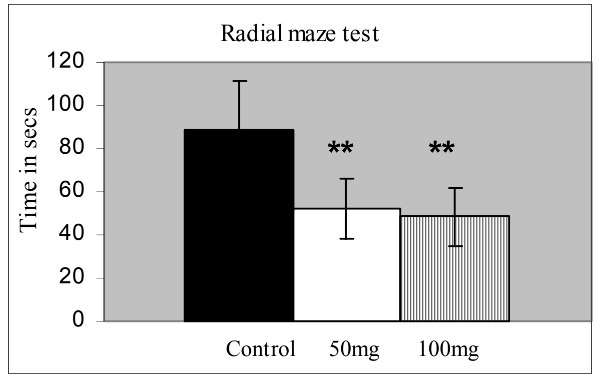


**Fig. 5**: Effect of TRP supplementation (50 mg/kg and 100 mg/kg b.w) on memory function in rats in a radial maze. Values are mean ± S.D (n=6). Significant difference by Newman Keuls test; **P<0.01 vs respective control group.

enter the arm, which was baited with food reward. The time elapsed before the rat entered the baited arm was recorded in testing session.

***Statistical analysis***

Data are presented as mean ± SD. Neurochemical and behavioral data were analyzed by 1 WAY ANOVA. Individual comparision were made by Newman-Keuls test. A value of $P < 0.05$ was considered significant.

## RESULTS

Oral administration of TRP at both doses (50 mg/kg and 100 mg/kg) for 6 weeks significantly increased the plasma TRP 61% and 64% respectively (P<0.01), brain TRP 33% and 38% respectively (P<0.01), 5HT 444% and 398% respectively (P<0.01) and 5HIAA 71% and 46.7% respectively (P<0.05) (fig. 3).

A significant improvement in memory function of rats following treatment with TRP was also observed at both doses when monitored in the radial maze (P<0.01) and passive avoidance test (P<0.05). A significant increase (p<0.05) in time taken to enter the dark (punishable) compartment was exhibited by TRP treated rats compared to control rats (fig. 4). An alteration of performance in TRP treated rats was also observed in the Radial maze. A significant decrease (p<0.01) in time to enter the baited arm was exhibited by TRP treated rats (fig. 5).

## DISCUSSION

As expected the administration of TRP at doses 50 mg/kg and 100 mg/kg significantly increased plasma TRP levels and brain 5HT metabolism in female rats. Previous studies in humans and animals also show that brain 5HT synthesis is influenced by the supply of TRP to the brain (Young *et al*., 1989; Kimbrough *et al*., 1992). Under normal conditions, the brain enzyme TRP hydroxylase is only 50% saturated therefore an increase in TRP will tend to automatically increase brain serotonin production (Hamon *et al*., 1981; Young and Gauthier, 1981). Serotonin plays a significant role in learning and memory processes but data on the effect of 5HT on these processes are inconsistent. There are reports that directly relate increased brain TRP and 5HT with enhanced cognitive performances (Levkovitz *et al*., 2003; Laercio *et al*., 2004) and depletion of brain TRP and 5HT with impairment in memory (Lieben *et al*., 2004; Harrison *et al*., 2004). However, research also shows enhanced learning following TRP depletion (Normile *et al*., 1990). Our finding of enhanced cognitive performance in rats following long-term TRP administration support the contention that brain 5HT and TRP levels have a positive effect on memory process (Markus *et al*., 2002; Laercio *et al*., 2004).

In current study we chose the radial arm maze and passive avoidance test to measure the changes in memory function following long term TRP administration. It has been demonstrated in number of experiments that rat showed rapid learning of spatial discrimination and excellent memory for the spatial location of different quantities and qualities of food on the arm of the maze. The passive avoidance test was chosen because it has been reliably shown to depend on the actual inhibition of one particular response (stepping with four paws on the grid). We found a significant improvement in the memory of rats given TRP for 6 weeks. This study demonstrates that administration of TRP affects the performance of rats on the RAM. Spatial working memory was enhanced in TRP treated rats. Compared to control the TRP treated rats took less time to enter the correct (baited) arm. Results of passive avoidance test also exhibited an improvement in retention of passive avoidance learning in rats. TRP treated rats took more time to enter the dark (punishable) box. This enhancement in the cognitive performance of TRP treated rats seemed dose dependent. The decrease in time at the dose of 50 and 100 mg/kg was 42% and 48% respectively in radial arm maze and the increase in time in passive avoidance test at 50 and 100 mg/kg was 34% and 52% respectively.

In conclusion our results suggest that increase in brain 5HT following administration of TRP may be responsible for the improved cognitive performance in TRP treated rats. Significant results obtained from RAM and passive avoidance test suggest that the both methods may be useful in determining the effect on memory of rats and may also be helpful in determining the neurochemical mechanism involved in memory function. The present enhancement in memory function following long term TRP administration also suggests the use of serotonin precursor tryptophan as a drug to improve memory function in various memory disorders.

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