

Dose-dependent effects of tryptophan on learning and memory

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Abstract: The concentration of 5-hydroxytryptamine (5-HT, Serotonin) varies as a result of physiological changes in the availability of its precursor tryptophan to the serotonergic neurons in the brain. Increase in brain tryptophan occurs following an increase in plasma tryptophan concentration. Tryptophan intake increases brain serotonin metabolism and enhances memory. The Present study was designed to investigate the effects of oral administration of tryptophan (TRP) at different doses (100, 300 and 500mg/kg) for two weeks on learning and memory functions and Neurochemical changes in rats. Control rats were given drinking water. Assessment of memory in rats was done by using the water Maze. on the 14th day trail training of water Maze was given to rats and after 1h of this 2nd trial of these rats were done. On the next day (After 24h of trail) long-term memories of these rats were monitored. After 1 hour of this all rats were killed by decapitation using guillotine. Brain and blood was collected and stored at -70°C. Neurochemical estimations of Plasma and brain tryptophan, 5-HT and 5-HIAA in brain were made by HPLC-EC. Result showed that administration of tryptophan enhanced performance on water Maze test. Tryptophan treated animals exhibited higher level of Plasma as well as brain tryptophan. 5-HT and 5-HIAA levels were also increased in tryptophan treated rats. Findings are discussed in context with the role of 5-HT metabolism in learning and memory process in rats. Results may help to understand the 5-HT changes following long term TRP administration in a dose dependent manner and will help to suggest the use of TRP in serotonin related illnesses.

Keywords: 5HT, memory, Morris water maze, tryptophan.

INTRODUCTION

Tryptophan, an essential amino acid, is the precursor of neurotransmitter 5-hydroxytryptamin (5-HT; serotonin) (Gal *et al.*, 1963). Tryptophan loading increases plasma tryptophan/ large neutral amino acids (TRP/LNAA) ratio and increases brain serotonin activity (Haleem, 1999). Whereas, acute tryptophan depletion lowers plasma tryptophan ratio (TRP/LNAA) and decreases brain serotonin activity (Rutten *et al.*, 2007). Apart from other physiological functions, 5-HT also enhances cognitive performance. Hughes *et al.* (2002) have reported impair cognition upon decreasing brain 5-HT levels. Impaired cognitive performance is observed upon chronic lowering of serotonin (Jenkins *et al.*, 2010). Memory functions are reported to be impaired by 5-HT deficits and vice versa (Porter *et al.*, 2003).

It had been shown previously that tryptophan at the doses of 50- and 100 mg/kg, increases plasma TRP levels and brain 5-HT metabolism in female rats, thereby improving their cognitive performance (Khaliq *et al.*, 2006). Increased serotonergic levels may also play an important role in the attenuation of addictive effects of drugs of abuse (Ikram and Haleem, 2011) and this may be associated with an alteration of cognitive process/es during experience. Gender- as well as testing paradigm may affect cognitive performances. Water maze and

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radial maze tasks differentially tax motivation (the water maze is aversive and the radial maze is appetitive) and motor systems (running versus swimming), which could produce different results even among control groups (Ormerod and Beninger, 2002). Sex related differences of raphe-hippocampal serotonin neurotransmission have been reported (Haleem, 2011), suggesting different cognitive performance/other functions in male and female subjects. Since spatial learning in general and Morris water maze performance in particular appear to depend upon the coordinated action of different brain regions and neurotransmitter systems constituting a functionally integrated neural network (D'Hooge and De Deyn, 2001). We selected Morris water maze test as for monitoring the effects of various doses of tryptophan on learning and memory.

The present study was designed to test the effects of long-term oral administration of TRP on memory function in male rats by Morris water maze test.

MATERIALS AND METHODS

Animals

Locally bred Albino Wistar weighing 200-250 grams were used for the experiment. They were caged individually in plastic cages with free access to cubes of standard rodent diet and tap water for 2 days before starting the experiment.

Drug administration

Tryptophan purchased from Merck (Germany), was dissolved in tap water and given at doses of 100, 300 and 500mg/kg for two weeks orally to test rats. For oral administration a small stainless steel feeding tube fixed to a 1ml syringe was used. An equal amount of water was given orally to control rats.

Experimental protocol

24 rats were divided into four groups each containing 6 animals: (i) Water-, (ii) Tryptophan (100mg/kg)-, (iii) Tryptophan (300mg/kg)- and (iv) Tryptophan (500mg/kg) administered animals. Tryptophan was administered in test animals orally (once a day, for two weeks). Water was administered orally to the control rats. On day 14, Morris Water Maze test training was done 1hr post tryptophan administration. Short term memory was monitored 1hr post training, while long term memory was monitored 24hr post tryptophan administration (day 15). On day 15, rats were decapitated. Brain and plasma samples were collected and stored -70°C until analysis by HPLC-EC. All animal experiments, approved by the Institutional Ethics and animal Care Committee, were performed in strict accordance with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). All treatments and behavioral monitoring were done in a balanced design to avoid order and time effect (Haleem *et al.*, 2013).

Behavioral tests

Water maze test

The effects of long-term administration of tryptophan on spatial memory were examined by assessing performance in the Morris water maze. The water maze apparatus used in the present study consisted of a black tank, 210cm in diameter and filled to a level that was 2cm higher than the platform height. Water temperature was at room temperature, 21±1°C. The platform (10 cm×10 cm) was made of clear acrylic and was hidden 2cm below the surface of water in a fixed location (Williams *et al.*, 2002). Water was made opaque by adding milk to it. The experiment was performed after two weeks of daily administration of tryptophan. Initially the rats were trained and during the training session each rat was placed into the water facing the wall of the tank and allowed 120 seconds to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 second. If it failed to find the platform within the allowed time it was guided gently onto the platform. Rats were tested for short-term memory (STM) 60 minutes after training and finally for long-term memory 24 hours later. The STM and LTM were determined by recording the retention latency (RL). Retention Latency is the time taken by each rat to locate the hidden platform after 1 hour and 24 hour of training. The cut off time for each session was 2 minutes.

Brain dissection

After decapitation, skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70°C for neuro chemical estimations using High performance liquid chromatography with electrochemical detection (HPLC-EC) (Ikram *et al.*, 2012; Mirza *et al.*, 2013).

Neuro chemical estimations by HPLC-EC

HPLC-EC determination was carried out as described earlier (Ikram *et al.*, 2007; Ikram *et al.*, 2011; Ikram *et al.*, 2010). A 5μ Shim-pack ODS separation column of 4.0 mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer of pH 2.9 at an operating potential of 2000-3000 psi on Shimadzu HPLC pump. Electrochemical detection was achieved on Shimadzu LEC 6A detector at an operating potential of +0.8V.

STATISTICAL ANALYSIS

Results are represented as means ± SD. Neuro chemical and behavioral data were analyzed by one-way ANOVA. Post-hoc analysis was done Newman-Keul's test. Values of p<0.05 were considered significant.

RESULTS

Fig. 1 shows basal values of animals in the Morris water maze training before assigning them to various groups. Values of different groups were not statistically different from each other. Data analyzed by one-way ANOVA showed non-significant effect of TRP supplementation (df= 3,20; F= 0.1201) on training of rats.

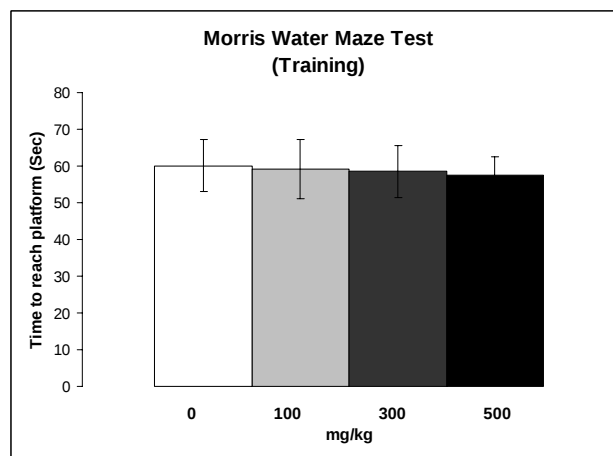


Fig. 1: Morris Water Maze test training session. Values are means ± S.D (n=6). Individual differences among groups were not significant.

Fig. 2 shows the effect of TRP supplementation on Morris water maze test. Data on short term memory (fig. 2a), as analyzed by one-way ANOVA showed significant effect of repeated TRP supplementation on short-term memory ($df=3, 20$; $F=26.131$, $p<0.01$). Post hoc analysis by Newman-Keuls test showed that administration of TRP at all three doses significantly enhanced ($p<0.01$) short-term memory as compared to water treated controls. However, Effect was more pronounced at 300mg/kg dose. Data on long term memory (fig. 2a), as analyzed by one-way ANOVA showed significant effects of repeated of repeated on long-term memory ($df=3, 20$; $F=57.867$, $p<0.01$). Post hoc analysis by Newman-Keuls test showed that administration of TRP at all three doses significantly enhanced ($p<0.01$) long-term memory as compared to water treated controls. However, Effect was more pronounced at 300mg/kg dose.

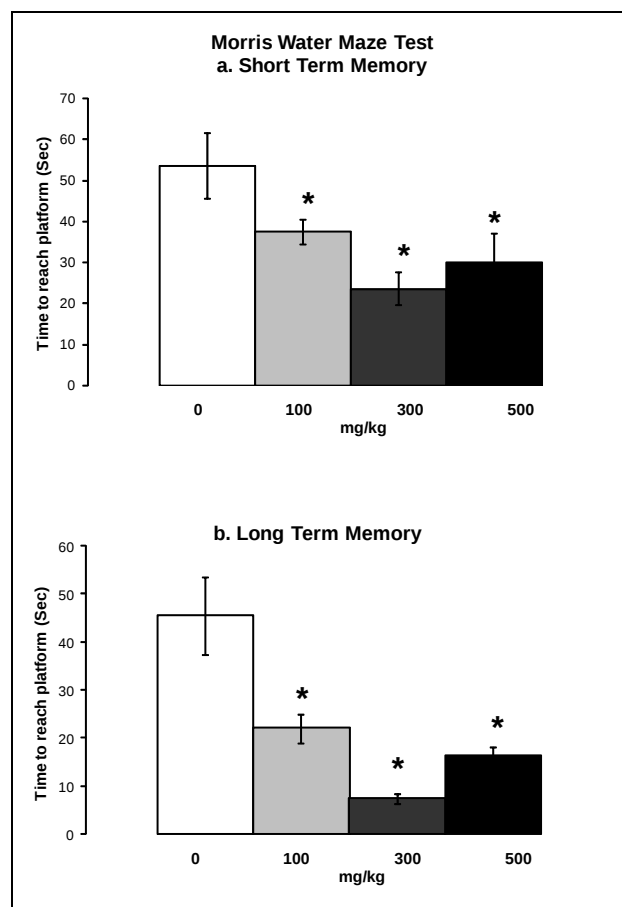


Fig. 2: Effects of low, moderate and high doses of tryptophan (100, 300 & 500mg/kg) on Morris water maze activity in rats. Values are means $\pm$ SD. Significant differences by Newman-Keuls test: * $p<0.01$ from water treated controls following one-way ANOVA.

Fig. 3 shows the effect of TRP supplementation on plasma and brain tryptophan levels. Data on plasma tryptophan levels (fig. 3a), as analyzed by one-way ANOVA showed significant effect of repeated TRP

supplementation on plasma TRP levels ($df=3, 20$; $F=93.384$, $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that TRP administration at all three doses significantly increased ($p<0.01$) plasma TRP levels in rats. Analysis of the data on brain tryptophan levels (fig. 3b), by one-way ANOVA showed significant effect of repeated TRP supplementation on brain TRP levels ($df=3, 20$; $F=89.737$, $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that TRP administration at all three doses significantly increased ($p<0.01$) brain TRP levels in rats.

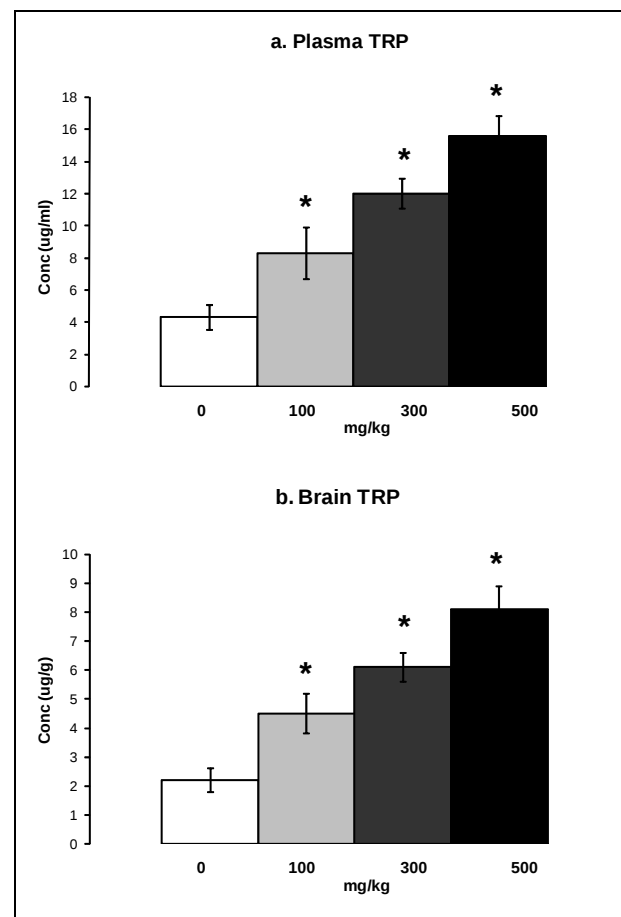


Fig. 3: Effects of low, moderate and high doses of tryptophan (100, 300 & 500mg/kg) on (a) plasma- and (b) brain tryptophan levels in rats, as analyzed by HPLC-EC. Values are means $\pm$ SD. Significant differences by Newman-Keuls test: * $p<0.01$ from water treated controls following one-way ANOVA.

Fig. 4 shows the effect of TRP supplementation on brain 5-HT and 5-HIAA levels. Data on 5-HT levels (fig. 4a), as analyzed by one-way ANOVA showed significant effect of repeated TRP supplementation on 5-HT levels ($df=3, 20$; $F=35.985$, $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that TRP administration at all three doses significantly increased ($p<0.01$) 5-HT levels in rats. Analysis of the data on 5-HIAA levels (fig. 3b), by one-way ANOVA showed significant effect of repeated

TRP supplementation on brain 5-HIAA levels ($df=3, 20$; $F=43.00$, $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that TRP administration at all three doses significantly increased ($p<0.01$) 5-HIAA levels in rats.

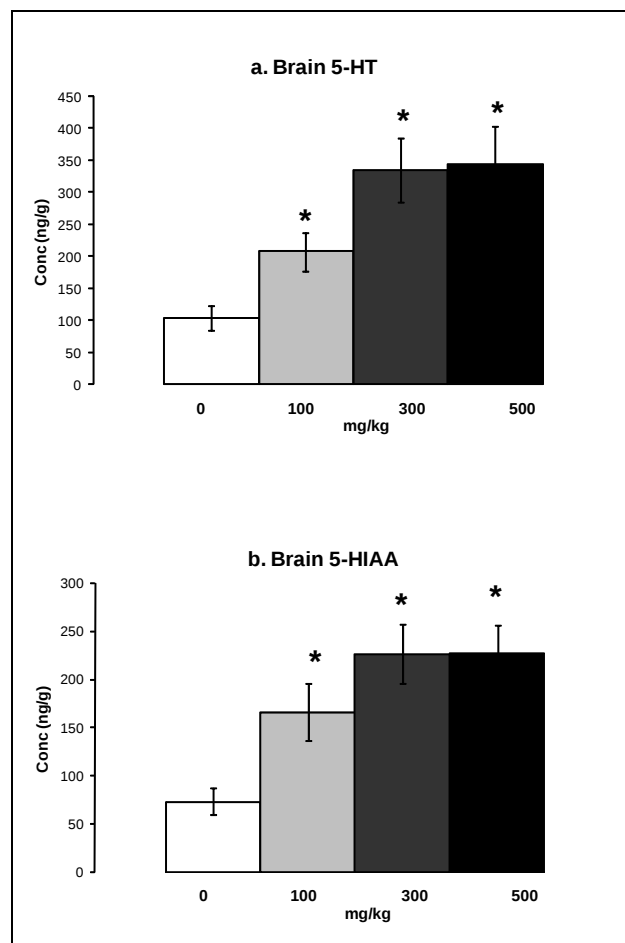


Fig. 4: Effects of low, moderate and high doses of tryptophan (100, 300 & 500mg/kg) on brain (a) 5-HT and (b) 5HIAA levels in rats, as analyzed by HPLC-EC. Values are means $\pm$ SD. Significant differences by Newman-Keuls test: * $p<0.01$ from water treated controls following one-way ANOVA.

DISCUSSION

Results from present study show an improvement in the cognitive performance at all three doses (fig. 2). However, this improvement was more pronounced at moderate dose (300 mg/kg) and further increase in dose (500 mg/kg) did not improve cognitive performance. This was in accordance with the brain 5-HT and 5-HIAA levels which were increased in a dose dependent manner till 300 mg/kg but not any further (fig. 4), despite the elevated levels of TRP in plasma and brain at the dose of 500 mg/kg (fig. 3). Tryptophan is an essential amino acid; a precursor of neurotransmitter serotonin (Markus *et al.*, 2002). Oral supplementation of tryptophan increases brain 5-HT activity (Markus *et al.*, 2002). Administration of

TRP increases brain 5-HT metabolism (Haleem, 1990), because the rate limiting enzyme in the synthetic pathway of 5-HT, tryptophan hydroxylase is unsaturated with its substrate.

Improvement in the cognitive functions (fig. 2) might be due to the increased synthesis of 5-HT as a result of increased supply of TRP to the brain (fig. 3). Decreasing brain 5-HT levels has been shown to impair cognition, possibly by decreasing 5-HT, as decreased brain 5-HT levels also impair cognition (Riedel *et al.*, 2003; Porter *et al.*, 2003; Haider *et al.*, 2006). Acute tryptophan depletion has a negative effect on memory consolidation (Schmitt *et al.*, 2000). Memory impairment in the object recognition test (ORT) has been observed in rats after acute tryptophan depletion (Lieben *et al.*, 2004), whereas increased brain 5-HT activity is suggested to improve cognitive performance (Markus *et al.*, 2002). Our findings are in line with the previous studies and support the notion that increased brain TRP and 5-HT levels enhance the memory and cognitive performance.

In the present study we tested animals in Morris water maze. The performance in the Morris water maze has been reported to be related to the neurotransmitter systems and drug effects (McNamara RK, Skelton, 1993). MWM performance has been linked to long-term potentiation (LTP), making it a key technique in the investigation of hippocampal circuitry (Morris *et al.*, 1986). It has been used widely for testing the spatial or place learning. This enhancement in the cognitive performance of TRP treated rats seemed dose-dependent initially. However, it did not increase further at 500mg/kg (fig. 2). This indicates that the high dose of TRP which we used in the present study (500 mg/kg) is greater than the effective dose. This might be due to the reason that TRP was maximally absorbed at 300mg/kg and a greater dose (500mg/kg) did not affect its absorption any more.

In conclusion we suggest that increased performance of rats in the Morris water maze was due to increased brain TRP and 5-HT. Results obtained from this study may be helpful to use tryptophan as a therapeutic agent in memory disorders.

REFERENCES

- D'Hooge R and De Deyn PP (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Res. Brain Res. Rev.*, **36**(1): 60-90.
- Gal EM, Pockzick M and Marshall FD Jr (1963). Hydroxylation of tryptophan to 5-hydroxy tryptophan by brain tissue. *Biochem. Biophys. Res. Commun.*, **12**(1): 39-43.
- Haider S, Khaliq S, Ahmed SP and Haleem DJ (2006). Long-term tryptophan administration enhances cognitive performance and increases 5HT metabolism

- in the hippocampus of female rats. *Amino Acids*, **31**: 421-425.
- Haleem DJ (1999). Attenuation of 8-OH-DPAT- induced increases in 5-HT synthesis in brain regions of rats adapted to a repeated stress schedule. *Stress*, **3**(2): 123-129.
- Haleem DJ (1999). Serotonergic mechanism of antidepressant action and adaptation to stress. *J. Coll. Phy. Surg. Pak.*, **9**: 139-146.
- Haleem DJ (2011). Raphe-Hippocampal Serotonin Neurotransmission In: The sex related differences of adaptation to stress: Focus on serotonin-1A receptor. *Curr. Neuropsychopharmacol.*, **9**(3): 512-521.
- Haleem DJ, Ikram H, Haider S, Parveen T and Haleem MA (2013). Enhancement and inhibition of apomorphine-induced sensitization in rats exposed to immobilization stress: Relationship with adaptation to stress. *Pharmacol. Biochem. Behav.*, **112**(1): 22-28.
- Hughes JH, Gallager P and Young AH (2002). Effects of acute tryptophan depletion on cognitive functions in euthymic bipolar patients. *Eur. Neuropsychopharmacol.*, **12**(2): 123-128.
- Ikram H, Ahmed S and Haleem DJ (2011). Effects of Apomorphine on Loco motor Activity and monoamine metabolism: A dose related study. *Pak. J. Pharm. Sci.*, **24** (3): 315-321.
- Ikram H, Choudhry AM and Haleem DJ (2012). Regional neuro chemical profile following development of apomorphine-induced reinforcement. *Pak. J. Pharm. Sci.*, **25**(3): 513-519.
- Ikram H and Haleem DJ (2010). Haloperidol-induced tardive dyskinesia: Role of 5HT_{2C} receptors. *Pak. J. Sci. Ind. Res.*, **53**(3): 136-145.
- Ikram H and Haleem DJ (2011). Attenuation of apomorphine-Induced sensitization by buspirone. *Pharmacol. Biochem. Behav.*, **99**(3): 444-450.
- Ikram H, Samad N and Haleem DJ (2007). Neuro chemical and behavioral effects of m-CPP in a rat model of tardive dyskinesia. *Pak. J. Pharm. Sci.*, **20**(3): 188-195.
- Jenkins TA, Elliott JJ, Ardis TC, Cahir M, Reynolds GP, Bell R and Cooper SJ (2010). Tryptophan depletion impairs object recognition memory in the rat: Reversal by risperidone. *Behav. Brain Res.*, **208**(2): 479-483.
- Khaliq S, Haider S, Ahmed SP, Tahira Perveen T and Haleem DJ (2006). Relationship of brain tryptophan and serotonin in improving cognitive performance in rats. *Pak. J. Pharm. Sci.*, **19**(1): 11-15.
- Lieben CK, Van-Oorsouw K, Deutz NEP and Blokland A (2004). Acute tryptophan depletion induced by a gelatin-based mixture impairs object memory but not affective behavior and spatial learning in rat. *Behav. Brain Res.*, **15**(1): 53- 64.
- Markus CR, Olivier B and de Haan EH (2002). Why protein rich in alpha-lact albumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects raises brain serotonin activity, reduces cortisol concentration and improve mood under stress. *Am. J. Clin. Nutr.*, **71**(6): 1536-1544.
- McNamara RK and Skelton RW (1993). The neuro pharmacological and neuro chemical basis of place learning in the morris water maze. *Brain Res. Rev.*, **18**(1): 33-49.
- Mirza B, Ikram H, Bilgrami S, Haleem DJ and Haleem MA (2013). Neuro chemical and behavioral effects of green tea (camellia sinensis): A model study. *Pak. J. Pharm. Sci.*, **26**(3): 511-516.
- Morris RGM, Anderson E, Lynch GS and Baudry M (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate antagonist, AP5. *Nature*, **329**(6056): 774-776.
- Ormerod BK and Beninger RJ (2002). Water maze versus radial maze: Differential performance of rats in a spatial delayed match-to-position task and response to scopolamine. *Behav. Brain Res.*, **128**(2): 139-152.
- Porter RJ, Lunn BS and O'Brien JT (2003). Effects of acute tryptophan depletion on cognitive functions in Alzheimer's disease and in the healthy elderly. *Psychol. Med.*, **33**(1): 41-49.
- Riedel WJ, Sobczek S and Schmitt JA (2003). Tryptophan modulation and cognition. *Adv. Exp. Med. Biol.*, **527**: 207-213.
- Rutten K, Lieben C, Smiths L and Blokland A (2007). The PDE4 inhibitor rolipram reverses object memory impairment induced by acute tryptophan depletion in the rat. *Psychopharmacology*, **192**(2): 275-282.
- Schmitt JA, Jorissen BL, Sobczak S, Bostel MP, Hogervorst P, Deutz NE and Riedel WJ (2000). Tryptophan depletion impairs memory consolidation but improves focused attention in healthy young volunteers. *J. Psychopharmacol.*, **14**(1): 21-29.
- Williams MT, Morford LL, McCrea AE, Wood SL and Vorhees CV (2002). Administration of d, l- fenfluramine to rats produces learning deficits in the Cincinnati water maze but not the Morris water maze: relationship to adrenal cortical output. *Neurotoxicol. Teratol.*, **24**(6): 783-796