Altered brain serotonergic neurotransmission following caffeine withdrawal produces behavioral deficits in rats

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Abstract: Caffeine administration has been shown to enhance performance and memory in rodents and humans while its withdrawal on the other hand produces neurobehavioral deficits which are thought to be mediated by alterations in monoamines neurotransmission. A role of decreased brain 5-HT (5-hydroxytryptamine, serotonin) levels has been implicated in impaired cognitive performance and depression. Memory functions of rats were assessed by Water Maze (WM) and immobility time by Forced Swim Test (FST). The results of this study showed that repeated caffeine administration for 6 days at 30 mg/kg dose significantly increases brain 5-HT ($p<0.05$) and 5-HIAA ($p<0.05$) levels and its withdrawal significantly ($p<0.05$) decreased brain 5-HT levels. A significant decrease in latency time was exhibited by rats in the WM repeatedly injected with caffeine. Withdrawal of caffeine however produced memory deficits and significantly increases the immobility time of rats in FST. The results of this study are linked with caffeine induced alterations in serotonergic neurotransmission and its role in memory and depression.

Keywords: Caffeine, serotonin, depression, memory.

INTRODUCTION

Caffeine is widely consumed in beverages to obtain mild stimulant effect (Valzelli and Bernasconi, 1973; Tarnopolsky, 1994). It is considered as a drug of abuse as it is widely used drug. Caffeine usefulness is limited because caffeine withdrawal and its intake at larger doses precipitate unpleasant states including anxiety, decreased performance, headache, dysphoria and depression (Hughes *et al.,* 1991; Silverman *et al.,* 1992; Cacciatore *et al.,* 1996; Nehlig, 2010). Neurochemical studies reported an increase in brain concentration of TRP, 5-HT and its metabolite 5-HIAA in rats following caffeine administration (Corrodi *et al.,* 1972; Fernstrom *et al.,* 1984; Hadfield and Milio, 1989; Kirch *et al.,* 1990; Haleem *et al*., 1995). Caffeine withdrawal results in decreased serotonergic metabolism (Haleem *et al.,* 1995).

Caffeine administration is reported to improve learning and memory (Lieberman *et al.,* 2002; Prediger *et al.,* 2005; Cunha and Agostinho, 2010) and acute withdrawal of caffeine has been reported to impair cognition (Bernstein *et al.,* 1998; Rogers *et al.,* 2005). It was also reported that caffeine has no memory enhancing effect (Kyle *et al*., 2010). Memory functions have been positively correlated with 5HT content in the brain. Increased 5-HT neurotransmission is known to improve memory function (Haider *et al.,* 2006; Khaliq *et al.,* 2006; Wilkosc *et al.,* 2010) and decreased 5-HT is known to impair memory in human (Porter *et al.,* 2003; Schmitt *et al.,* 2006) and animals (Haider *et al.,* 2005). Alterations in serotonin levels and neurotransmission are also associated with depressive disorders. Role of 5HT in depression is well documented (Owens and Nemeroff, 1994). Decreased brain 5HT has been associated with depression (Greden, 1974).

Previous study on caffeine in our laboratory showed alterations in the serotonergic neurotransmission suggesting that this may cause depression in condition of caffeine withdrawal (Haleem *et al.,* 1995). The study is an extension of the previous study and was designed to find the effects of repeated caffeine intake and its withdrawal on memory function and depression in rats and its relationship with serotonergic metabolism.

MATERIALS AND METHODS

Animals

In this study eighteen locally bred albino Wistar rats (180-200g) was used. They were housed individually in their home cages under a 12 h light-dark cycle and at controlled room temperature (22 ± 2°C). They were given standard rodent diet and tap water for at least 3-4 days before experimentation. All experiments were performed according to a protocol approved by Local Animal Care Committee.

Experimental Procedure

Animals were divided into control and 2 test groups. Control animals were injected with saline for 6 days.

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Caffeine at a dose of 30 mg/kg was administered intraperitoneally to the 1st test group for 6 days. 2nd test group was injected with the same dose of caffeine for 5 days. After 5 days of caffeine administration, on the 6th day saline was injected to these rats to monitor the withdrawal effect of caffeine. After half hour of post injection, behavioral tests were carried out for the assessment of memory and depression. Rats were decapitated on 6th day to collect the brain samples. All experiments were performed in a balanced design to avoid the order effect. Rat's brain samples were taken out from the cranial cavity within 30 seconds of decapitation. Fresh brains were dipped in chilled saline and they were stored at low temperature (-70°C) until the analysis of 5-HT and 5-HIAA by HPLC-EC.

Neurochemical Estimations

Analysis of 5-HT and 5-HIAA levels was carried out by HPLC-EC (Haleem and Haider, 1996). A 5-II Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Mobile phase contains methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer at pH 2.9.Operating pressure was 2000-3000psi and Schimadzu LEC 6A detector of 0.8 volts was used.

Behavioral tests

Water Maze Test

The effect of caffeine intake and its withdrawal on memory was examined by assessing performance in a Water Maze task, which is the classic experimental procedure (Morris, 1981). The WM apparatus used in this study is a rectangular glass tank (60 x 30cms) filled with milky water (depth of water is 15cm). A wooden platform (15 x 13cms) was hidden 2cm below the surface of water in a fixed location. Initially the rats were trained and during this session rats were placed in the water for 120 seconds so they can locate the submerged platform. When they find the platform they were allowed to stay there for 10 seconds so that they can memorize the location of platform. Memory performance of rats was tested 60 minutes after the training session. The effect on memory was determined by noting the latency time during the test session (time taken by the rat to locate the hidden platform). The time for test session was 120 seconds.

Forced Swim Test

Forced swim testing and measurement of immobility

Forced swimming test is a widely used model for analyzing depression like behavior in rats and mice (Porsolt *et al.*, 1977). Rats were placed in an inescapable chamber filled with water. During the test session the swimming behavior was noted for 360 seconds. The rats were individually forced to swim for 6 minutes in a glass tank (height 56 cm, width 20cm) which contained water to a height of 22cm at 25°C. The water height was such that the animal could not touch the bottom of the tank. The immobility time was calculated by taking the difference of total time and swimming time [360(s) – swimming time]. It is defined as the time when the rat makes no more attempts to escape.

STATISTICAL ANALYSIS

Results are presented as Means ± S.D. Data were analyzed by one way ANOVA; p value < 0.05 were considered significant.

RESULTS

Concentration of 5-HT and 5-HIAA were determined in the brain following caffeine administration at 30 mg/kg dose for 6 days. Analysis by one way ANOVA showed a significant effect following caffeine administration and its withdrawal on 5-HT ($F=13.48$; $p<0.01$) and 5-HIAA ($F=4.9$; $p<0.01$). Neuman Keul test showed that 5-HT and 5-HIAA levels were significantly increased following caffeine administration for 6 days. Withdrawal of the drug significantly ($p<0.05$) decreased 5-HT levels. Brain 5-HIAA levels following withdrawal exhibited a tendency of decrease which was however statistically not significant (fig. 1).

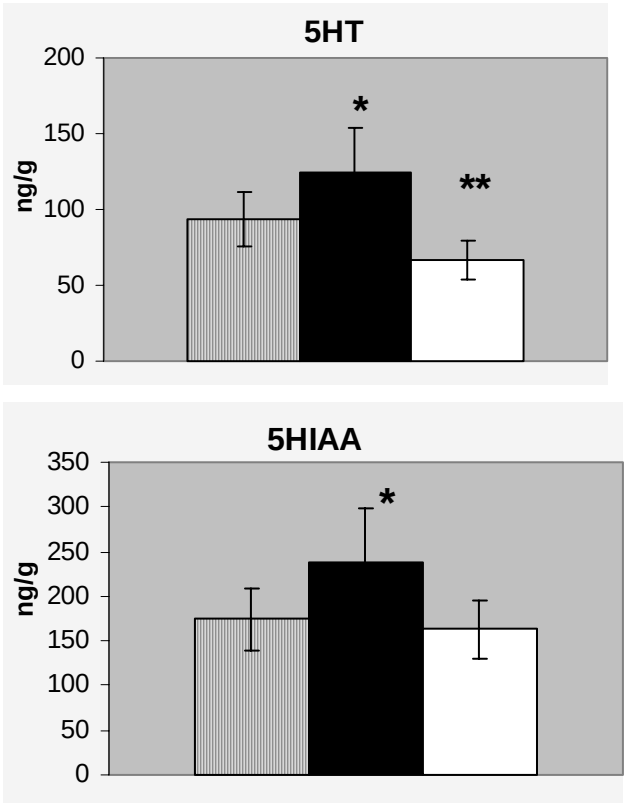

Fig. 1: Effect of caffeine administration (Black) and caffeine withdrawal (White) on brain 5-HT and 5-HIAA levels in rat. Values are mean ± S.D (n=6). Significant difference by Newman *K*euls test; *P<0.05; vs respective control group.

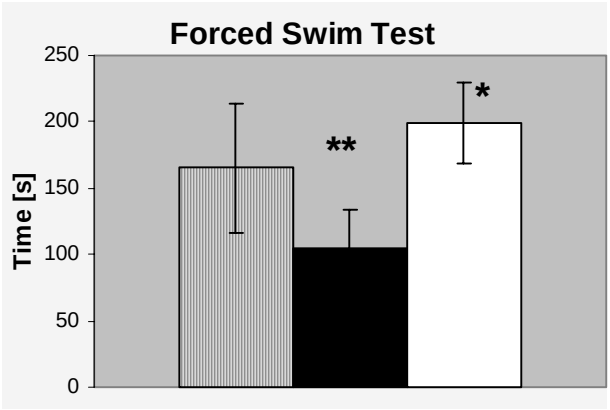

Fig. 2: Effect of caffeine administration (Black) and caffeine withdrawal (White) on immobility time of rats in FST. Values are mean ± S.D (n=6). Significant difference by Newman *K*euls test; *P<0.05, **P<0.01 vs respective control group.

Fig. 2 shows the effect of 30 mg/kg caffeine administration and withdrawal on immobility time in FST. One way ANOVA revealed a significant effect (F=14.53; p<0.01). Analysis by Neuman-Keuls test showed that the immobility time of rats given 30mg/kg for 6 days was significantly (p<0.01) smaller when compared to the control rats whereas the immobility time of rats after caffeine withdrawal was significantly increased (p<0.05).

Effect of caffeine administration and its withdrawal on memory was noted one hour after the training. Analysis by one way ANOVA showed a significant effect (F=15.7; p<0.01). Post hoc analysis showed that the memory of rats were significantly (p<0.05) improved following repeated caffeine administration whereas caffeine withdrawal significantly impaired (p<0.01) the memory of rats (fig. 3).

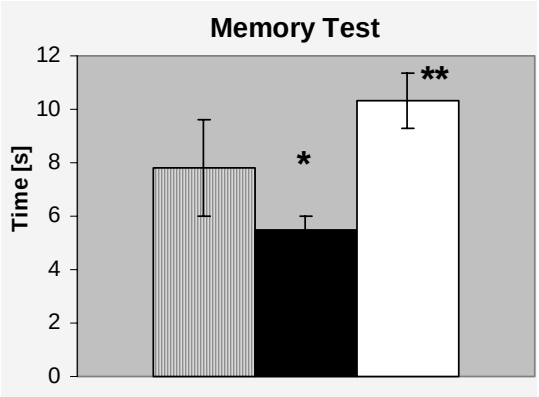

Fig 3: Effect of caffeine administration (Black) and caffeine withdrawal (White) on memory functions of rats in WM. Values are mean ± S.D (n=6). Significant difference by Newman *K*euls test; *P<0.05, **P<0.01 vs respective control group.

Fig. 4 shows the traces of HPLC-EC chromatograms showing separation of indoleamines in standard and in the brain extract of control rat, caffeine treated rat and caffeine withdrawal rat.

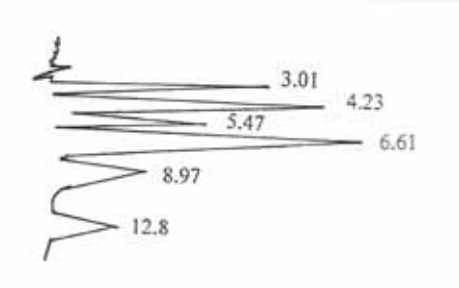

Fig. 4a: Trace of HPLC-EC chromatogram showing separation of indoleamines in a standard (Retention time of 5-HT and 5-HIAA is 12.8 min and 6.61 min respectively).

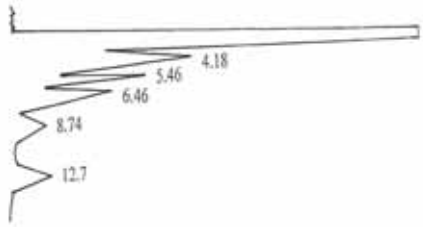

Fig. 4b: Trace of HPLC-EC chromatogram showing separation of indoleamines in the brain extract of control rat (Retention time of 5-HT and 5-HIAA is 12.7 min and 6.46 min respectively).

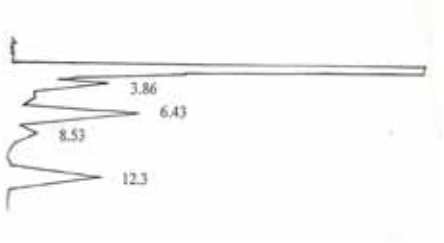

Fig 4c: Trace of HPLC-EC chromatogram showing separation of indoleamines in the brain extract of caffeine treated rat (Retention time of 5-HT and 5-HIAA is 12.3 min and 6.43 min respectively).

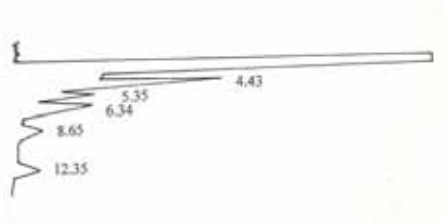

Fig. 4d: Trace of HPLC-EC chromatogram showing separation of indoleamines in the brain extract of caffeine withdrawal rat (Retention time of 5-HT and 5-HIAA is 12.35 min and 6.34 min respectively).

DISCUSSION

The main aim of this study was to examine the role of brain 5-HT on memory functions and depression following caffeine withdrawal in rats. Previous neurochemical studies reported a pronounced increase in brain 5-HT metabolism in rats after caffeine administration (Hadfield and Milio, 1989; Kirch *et al.*, 1990; Haleem *et al*., 1995). This study consistent with the previous reports showed that caffeine administered at 30 mg/kg dose increased brain levels of 5-HT and 5-HIAA. In addition to this we also report that 5-HT metabolism significantly decreased in condition of caffeine withdrawal. The main aim of this study was to examine the effects of caffeine administration and withdrawal on depression and memory function and their relationship with 5-HT metabolism in rats.

Caffeine is used as a social drink to improve the performance and elevate mood (Lieberman *et al.,*1987; Bernstein *et al.,* 1994; Rogers *et al*., 2005). It is the most commonly used drug in the world which is present in coffee, soft drinks, chocolates and candies. It is also known to increase the performance by delaying the onset of fatigue (Mactosh and Wright, 1995). It was reported previously that acute administration of caffeine increased brain levels of 5-HT and 5-HIAA (Corrodi *et al.,* 1972; Fernstrom *et al.,* 1984). This increase in 5-HT metabolism following caffeine administration may be involved in the improvement of memory functions. Studies on the role of serotonin in cognitive functioning have provided information for a serotonergic involvement in memory and attention (Khaliq *et al.,* 2006). It has been noted previously that increasing 5-HT metabolism has been known to improve cognitive processes (Haider *et al.,* 2006) whereas decrease in 5-HT levels is known to impair memory functions (Haider *et al.,* 2005; Schmitt *et al*., 2006). In the present study, caffeine administration at 30 mg/kg dose for 6 days significantly enhanced memory function which was observed in WM test. This increase in memory function after caffeine administration was linked to the increased 5-HT levels observed in this study. On the other hand caffeine withdrawal significantly impaired memory function as evidenced by the increased latency time in finding the platform in the WM test which correlates with the decreased 5-HT levels in brain.

5-HT is also known to have a role in depression (Owens and Nemeroff, 1994; Papolos *et al.,* 1996). Evidence exists suggesting low levels of 5-HT metabolism in depression (Haleem *et al.,* 1995). A decrease in 5-HT following caffeine withdrawal may be involved in depression (Greden, 1974; Bruce, 1990). In the present study, repeated caffeine administration for 6 days significantly increased depression like behavior in rats as seen in forced swimming test. Withdrawal of the drug from chronically caffeine treated animals caused a significant decrease in 5-HT metabolism and a simultaneous increase in immobility time in FST which confirm the role of serotonergic neurotransmission in the pathophysiology of depression. In conclusion, we report that behavioral deficits following caffeine withdrawal described here may contribute to the alterations in serotonergic neurotransmission.

REFERENCES

Bernstein GA, Carroll ME, Crosby RD, Perwien AR, Go FS and Benowitz NL (1994). Caffeine effects on learning, performance, and anxiety in normal school-age children. *J. Am. Acad. Child Adolesc. Psychiatry*, **33**(3): 407-415.

Bernstein GA, Carroll ME, Dean NW, Crosby RD, Perwien AR and Benowitz NL. (1998). Caffeine withdrawal in normal school-age children. *J. Am. Acad. Child Adolesc. Psychiatry*, **37**(8): 858-65.

Bruce MS. (1990). The anxiogenic effects of caffeine. *Postgard Med. J.*, **66**: 581-584.

Cacciatore R, Helbling A, Jost C and Hess B. (1996). Episodic headache, diminished performance and depressive mood. *Schweiz. Rundsch. Med. Prax.*, **85**(22): 727-729.

Corrodi H, Fuxem K and Jonsson. (1972) Effect of caffeine on central monoamines neurons. *J. Pharm. Pharmacol*., **24**(2): 155-158.

Cunha RA and Agostinho PM. (2010). Chronic caffeine consumption prevents memory disturbance in different animals models of memory decline. *J. Alzheimers Dis.* Ahead to print.

Fernstrom MH, Bazil CW and Fernstrom JD. (1984). Caffeine injection raises brain trypytophan level, but does not stimulate the rate of serotonin in rat brain. *Life Sci*., **35**(12): 1241-1247.

Greden JF (1974). Anxiety or caffeinism: A diagnostic dilemma. *Am. J. Psychiatry*, **131**: 1089-1092.

Hadfield MG and Milio C (1989) Caffeine and regional brain monoamine utilization in mice. *Life Sci*., **45**(26): 2637-2644.

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**:1-6.

Haider S, Shameem S, Ahmed SP, Perveen T and Haleem DJ (2005). Repeated administration of lead decreases brain 5HT metabolism and produces memory deficits in rats. *Cellular & Molecular Biology*, **10**: 669-676.

Haleem DJ and Haider S (1996). Food restriction decreases serotonin and its syntheses rate in the hypothalamus. *Neuroreport*, **7**: 1153-1156.

Haleem DJ, Yasmeen A, Haleem MA and Zafar A (1995). 24h withdrawal following repeated administration of caffeine attenuates brain serotonin but not tryptophan in rat brain: implications for caffeine induced depression. *Life Sci.*, **57**(19): 285-292.

Hughes JR, Higgins ST, Bickel WK, Hunt WK, Fenwick JW, Gulliver SB and Mireault GC (1991). Caffeine self-administration, withdrawal, and adverse effects among coffee drinkers. *Arch. Gen. Psychiatry,* **48**(7): 611-617.

Khaliq S, Haider S, Ahmed SP, 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.

Kirch DG, Taylor TR, Gerhardt GA, Benowitz NL, Stephen C and Wyatt RJ (1990). Effect of chronic caffeine administration on monoamine and monoaminines metabolite concentrations in brain. Neuropharmacology, **29**(6): 599-602.

Kyle J, Fox HC and Whalley LJ (2010). Caffeine, cognition and socioeconomic status. *J. Alzheimers,* ahead to print.

Lieberman HR, Wurtman RJ, Ernde CG, Roberto C, Coviella I.G (1987). The effect of low doses of caffeine on human performance and mood. *Psycho-pharmacology,* **92**: 308-312.

Lieberman HR, Tharion WJ, Shukitt-Hale B, Speckman KL and Tulley R (2002). Effects of caffeine, sleep loss, and stress on cognitive performance and mood during U.S. Navy SEAL training. *Psychopharmacology,* (Berl)., **164**(3): 250-261.

MacIntosh BR and Wright BM (1995). Caffeine ingestion and performance of a 1,500-metre swim. *Can. J. Appl. Physiol*., **20**(2): 168-177.

Morris RGM (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, **12**: 239-260.

Nehlig A (2010). Is caffeine a cognitive enhancer? *J. Alzheimers Dis.,* Ahead to print.

Owens MJ and Nemeroff CB (1994). Role of serotonin in the pathophysiology of depression: Focus on the serotonin transporter. *Clin. Chem.,* **40**(2): 288-295.

Papolos DF, Yu YM, Rosenbaum E and Lachman HM (1996). Modulation of learned helplessness by 5-hydroxytryptamine2A receptor antisense oligodeoxy-nucleotides. *Psychiatry Res*., **63**(2-3): 197-203.

Porsolt RD, Bertin A and Jalfre M (1977). Behavioral despair in mice: A primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.*, **229**(2): 327-236.

Porter R.J, 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.

Prediger RD, Pamplona FA, Fernandes D and Takahashi RN (2005). Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD) – The spontaneously caffeine hypertensive rats. *Int. J. Neuropsychopharmacol.*, **8**(4): 583-594.

Rogers PJ, Heatherley SV, Hayward RC, Seers HE, Hill J and Kane M (2005). Effects of caffeine and caffeine withdrawal on mood and cognitive performance degraded by sleep restriction. *Psychopharmacology (Berl).*, **179**(4): 742-752.

Schmitt JA, Wingen M, Ramaekers JG, Evers EA and Riedel WJ (2006). Serotonin and human cognitive performance. *Curr. Pharm. Des.,* **12**(20): 2473-2486.

Silverman K, Evans SM, Strain EC and Griffiths RR (1992). Withdrawal syndrome after the double-blind cessation of caffeine consumption. *N. Engl. J. Med.,* **327**(16): 1109-1114.

Tarnopolsky MA (1994). Caffeine and endurance performance. *Sports Med.,* **18**(2): 109-125.

Valzelli L and Bernasconi S (1973). Behavioral and neurochemical effects of caffeine in normal and aggressive mice. *Pharmacology Biochemistry and Behavior* , **1**(3): 251-254.

Wilkosc M, Hauser J, Tomaszewska M, Dmitrzak-Weglarz M, Skibinska M, Szczepankiewicz A and Borkowska A (2010). Influence of dopaminergic and serotonergic genes on working memory in healthy subjects. *Acta Neurobiol. Exp.* (Wars). **70**(1): 86-94.