



stimulus, found a significant increase in P300 amplitude and area 30 minutes after caffeine intake (8). There was no significant decrease in P300 latency. The reaction time in oddball paradigm was not significantly different before and after caffeine consumption. Deslandes et al investigated the effect of caffeine on visual P300 by administering 400 mg caffeine in gelatin capsules. They found a significant decrease in P300 latency at Fz (9). The amplitude of P300 showed a variable pattern of increase in some and decrease in some individuals.

It has been reported that the latency and amplitude of P3 in young Indian adults was comparable with age and sex matched subjects of the western world (10). Previous study from our lab has shown that substances like Vitamin E affects P3 (11).

Most of the studies on CNS effects of caffeine have been done using visual stimuli by administering caffeine either in gelatin capsules or in decaffeinated coffee with milk powder and sugar. In this study, we have administered pure caffeine (without decaffeinated coffee) with milk powder and sugar so as to rule out the effect of other constituents of coffee on CNS. A thorough literature search revealed scant work using auditory stimuli and this prompted us to evaluate the effect of caffeine on cognitive brain functions and motor response using auditory stimulus.

## METHODS

### Selection of subjects

The present study was conducted in the

Department of Physiology, University College of Medical Sciences, Delhi. The study group comprised of 40 normal, healthy young male undergraduate students in the age group of 18–25 years. The subjects had no history of head injury, epilepsy, hearing impairment, migraine, sleeping problems and drug abuse (nicotine, alcohol and opium). Due written consent was taken from them prior to the study. The subjects served as their own controls to minimize interindividual variation. Prior to the day of recording, the subjects were asked to abstain from caffeine containing substances for at least 12 hours and have adequate sleep.

### Administration of caffeine

Caffeine in the dose of 3 mg/kg body weight of the subject was given after mixing it with sugar and milk powder according to taste in 100 ml water.

### Recording of Event-related potentials

The recordings were done in a soundproof room before and 40 minutes after ingestion of caffeine. The subjects were given a trial session a day before the recording to familiarize them with the stimuli and the recording procedure. The ERPs were recorded on Nihon Kohden Neuropack  $\mu$  MEB 9100 using silver-silver chloride disk electrodes from Fz, Cz and Pz (active electrodes) with FPz as grounding electrode and A1 and A2 as the ear reference electrodes placed according to the 10–20 international system. The skin-electrode contact impedance was kept below 5 K $\Omega$ . The subjects were instructed to close their eyes to avoid blink artifacts.

The auditory ERPs were recorded using “oddball” paradigm wherein two stimuli (target and nontarget) were presented in a random order by headphones. The target stimulus was a 2 KHz sound with 20% occurrence and the nontarget 1 KHz with 80% occurrence. The auditory stimuli had a 10 msec rise/fall time, 100 msec duration and intensity of 60 dB above the hearing threshold. The responses were filtered with a band pass of 0.1–50 Hz and averaged for 30 responses. The analysis time was 100 msec before to 900 msec after the stimulus. The subjects pressed a button in response to the target stimulus. Data for 2 trials were obtained, stored and averaged by computer. The ERP peak latencies, baseline to peak amplitudes and reaction time were evaluated.

**Statistical analysis**

The data obtained were analyzed using SPSS software. The ERP amplitude data was normalized using log transformation as it was right skewed. Repeated measures ANOVA was done to compare the three electrodes at different places as per 10–20 electrode placement system before and after caffeine consumption. Multiple comparisons were obtained (within subjects comparison)

by Tukey Test at 5%. Paired t-Test was applied for analysis of Reaction Time.

**RESULTS**

The effect of caffeine on the latencies of N1, P2, N2 and P3 waves of ERPs are shown in Table I (Fig. 1). ANOVA showed a significant decrease in latency of P2 (P=0.044). However, Tukey failed to detect any significant decrease in the latency of P2 as the significance shown by ANOVA was

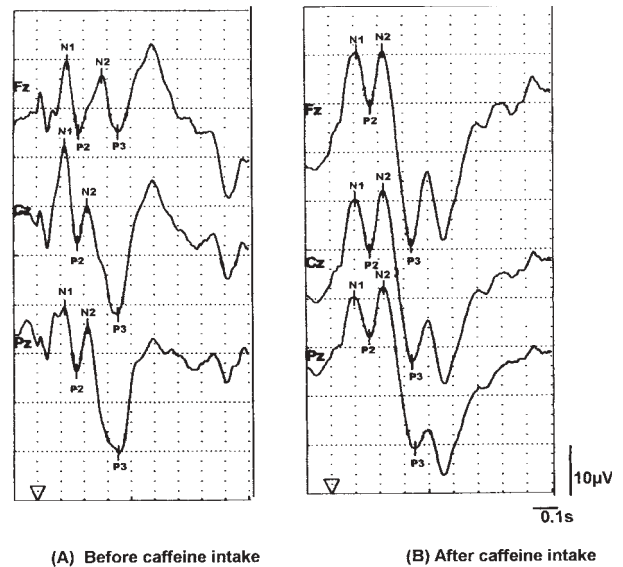


Fig. 1: Showing the effect of caffeine on ERPs before and after caffeine intake.

TABLE I: Showing the mean latencies (in msec) of ERP components at the three electrode positions, before and after caffeine intake (Mean±SD).

	Fz		Cz		Pz	
	Before	After	Before	After	Before	After
N1	110.73±14.54	107.13±14.49	100.43±11.57	98.15±12.91	95.15±11.41	92.77±12.52
P2	166.55±13.32	163.78±12.47*	160.88±11.62	158.78±10.41*	158.07±17.48	154.8±15.26*
N2	215.3±17.26	211.85±18.67	209.6±14.26	206.88±13.98	205.5±17.84	200.95±25.41
P3	320.12±32.15	318.72±30.57	317.82±39.37	310.47±33.4	318.45±32.35	313.98±30.52

\*P<0.05 when the baseline and after caffeine values are compared.

TABLE II: Showing the mean amplitudes (in  $\mu\text{V}$ ) of ERP components at the three electrode positions, following Log transformation, before and after caffeine intake.

	<i>Fz</i>		<i>Cz</i>		<i>Pz</i>	
	<i>Before</i>	<i>After</i>	<i>Before</i>	<i>After</i>	<i>Before</i>	<i>After</i>
N1	7.40	8.36	6.12	7.48	2.69	2.89
P2	3.12	3.20	2.99	4.00	3.09	4.13
N2	5.53	6.12	3.90	5.02	3.05	3.70
P3	4.16	6.63***	7.82	10.53***	14.40	17.07***

\*\*\* $P < 0.001$  when before and after caffeine intake values are compared.

marginal. The other waves namely N1, N2 and P3 showed a non-significant decrease in latency.

The mean amplitude of P3 increased significantly after caffeine intake. The amplitudes of all other ERP waves i.e. N1, P2 and N2 showed an increase but the change was not significant (Table II, Fig. 1).

The Reaction time was  $414.88 \pm 90.92$  msec before the subjects took caffeine. It decreased significantly ( $P=0.000$ ) to  $372.35 \pm 74.48$  msec after consumption of caffeine.

## DISCUSSION

The results of the present study revealed that there was a significant increase in the amplitude of P3 with a significant reduction in Reaction Time following caffeine consumption. Our finding of a non-significant decrease in P3 latency with a significant decrease in reaction time (RT) was similar to that of Seidl et al who had also used auditory oddball paradigm in 10 subjects (7). On the basis of known literature, they concluded that the stimulant

effect of caffeine was due to blocking of adenosine receptors. Kawamura et al used a dose of 500 mg of caffeine in 10 subjects and found a significant increase in the area and amplitude of P3, 30 minutes after caffeine intake (8). No significant change in P300 latency or reaction time was found. They suggested that caffeine affected the discriminating process involved in oddball paradigm. In another study, Lorist et al concluded that caffeine led to lower level of processing of irrelevant information which was evident by absence of P3b in ERPs elicited by irrelevant targets (6). There was a faster stimulus evaluation as evidenced by change in P3b peak latency.

Ruijter et al found an increase in P2 amplitude after caffeine and interpreted it as an arousal increasing effect (12). The RT was shorter with caffeine than with placebo. Ruijter et al concluded that caffeine led to increased information processing. In another study they found that caffeine had a general influence on early information processing and not specifically on spatial attention (13). Decrease in RT by caffeine has also been found by Azcona et al and Clubley et al (14, 15). Jacobson and Edgley found that a dose of 300 mg caffeine decreased RT and

movement time significantly. However, a dose of 600 mg showed no considerable change in RT and movement time (16). Caffeine has been found to shift EEG towards faster spectral components which has been interpreted as a reflection of elevated levels of energy (17).

Caffeine mainly acts by blocking adenosine receptors and thus bring about changes in the levels of various neurotransmitters like dopamine, adrenaline and glutamate. The A1 type of adenosine receptors have been linked to dopamine D1 receptors and A2A with dopamine D2 receptors (see 18). The A1 receptors have been found to be associated with regulation of alertness. Mesopontine cholinergic neurons are associated with regulation of arousal level and are under tonic A1 receptor control. Of various dopamine receptors isolated in brain, D1, D2, D4 and D5 have been found in hippocampus. Activation of D1 and D2 receptors in hippocampus has been found to improve acquisition and retention of working memory (19, 20). D1 and D2 receptors in prefrontal cortex of monkey have been reported to improve performance in working memory task (21). The abnormality in P3 in Parkinson's disease is perhaps due to changes in central dopaminergic mechanisms (22).

We therefore believe that the changes seen in the amplitude of P3 after caffeine intake could be due to modulation of

dopamine release in the cortex consequent to the inhibition of adenosine receptors by caffeine. The more positive going P3 was indicative of increased information processing by recruitment of cortical neurons after caffeine consumption. The effect of caffeine on latencies of ERPs was not significant. However, there was a significant decrease in RT brought about by caffeine. These findings suggested that rather than speeding up the central processes, caffeine accelerated motor (output) processes. Our findings of ERPs were similar to those obtained by various researchers using visual stimuli, thereby indicating that ERP changes were independent of the mode of stimuli given i.e. either pairing of two visual or two auditory stimuli for oddball paradigm. Thus it can be interpreted that caffeine ingestion directly influenced the cortical neuronal processes involved in higher functions like orientation, attention, alertness and motor response. This finding provides an electrophysiological evidence of caffeine induced improvement in cognitive and motor functions. Thus it may have relevance in patients with cognitive dysfunction as in Alzheimer's disease or motor impairment as in disorders like Parkinson's disease.

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#### REFERENCES

1. Fredholm BB. Astra Award Lecture. Adenosine, adenosine receptors and actions of caffeine. *Pharmacol Toxicol* 1995; 76(2): 93-101.
2. Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau E. Actions of caffeine in the brain with special reference to factors that contribute to its

- widespread use. *Pharmacol Rev* 1999; 51(1): 83–133.
3. Hollingsworth H. The influence of caffeine on mental and motor efficiency. *Arch Psych* 1912; 3: 1–16.
  4. Loke WH, Meliska CJ. Effects of caffeine use and ingestion on a protracted visual vigilance task. *Psychopharmacology* 1984; 84: 54–57.
  5. Lorist MM, Snel J, Kok A. Influence of caffeine on information processing stages in well rested and fatigued subjects. *Psychopharmacology* 1994 Jan; 113(3–4): 411–421.
  6. Lorist MM, Snel J, Kok A, Mulder G. Acute effects of caffeine on selective attention and visual search process. *Psychophysiology* 1996 Jul; 33(4): 354–361.
  7. Seidl R, Peryl A, Nicham R, Mauser E. A taurine and caffeine containing drink stimulates cognitive performance and well being. *Amino Acids* 2000; 19(3–4): 635–642.
  8. Kawamura N, Maeda H, Nakamura J, Morita K, Nakazawa Y. Effects of caffeine on event related potentials: comparison of oddball with single tone paradigms. *Psychiatry Clin Neurosci* 1996 Aug; 50(4): 217–221.
  9. Deslandes AC, Veiga H, Cagy M, Piedade R, Pompeu F, Ribeiro P. Effects of caffeine on the electrophysiological, cognitive and motor responses of the central nervous system. *Braz J Med Biol Res* 2005 Jul; 38(7): 1077–1086.
  10. Tandon OP. P3 event related evoked potential in young adults. *Indian J Physiol Pharmacol* 1990; 34(3): 191–194.
  11. Vaney N, Chouhan S, Bhatia MS, Tandon OP. Event related evoked potentials in Dementia: Role of Vitamin E. *Ind J Physiol Pharmacol* 2002; 46(1): 61–68.
  12. Ruijter J, DeRuijter MB, Snel J. The effects of caffeine on visual selective attention to color: An ERP study. *Psychophysiology* 2000; 37: 427–439.
  13. Ruijter J, de Ruyter MB, Snel J, Lorist MM. The influence of caffeine on spatial-selective attention: an event related potential study. *Clin Neurophysiol* 2000 Dec; 111(12): 2223–2233.
  14. Azcona O, Barbanoj MJ, Torrent J, Jane F. Evaluation of the central effects of alcohol and caffeine interaction. *Br J Clin Pharmacol* 1995; 40: 393–400.
  15. Clubley M, Bye CE, Henson TA, Peck AW, Riddington CJ. Effects of caffeine and cyclizine alone and in combination on human performance, subjective effects and EEC activity. *Br J Clin Pharmacol* 1979; 7: 157–163.
  16. Jacobson BH, Edgley BM. Effects of caffeine on Simple Reaction time and Movement time. *Aviat Space Environ Med* 1987; 58: 1153–1156.
  17. Hasenfratz M, Battig K. Acute dose-effect relationships of caffeine and mental performance, EEG, cardiovascular and subjective parameters. *Psychopharmacology (Berl)* 1994; 114: 281–287.
  18. Lorist MM, Tops M. Caffeine, fatigue and cognition. *Brain Cogn* 2003 Oct; 53(1): 82–94.
  19. Packard MG, White NM. Dissociation of hippocampus and caudate nucleus memory systems by post-training intracerebral injection of dopamine agonists. *Behav Neurosci* 1991; 105: 295–306.
  20. White NM, Packard MG, Seamans J. Memory enhancement by post-training peripheral administration of low doses of dopamine agonists: possible autoreceptor effect. *Behav Neural Biol* 1993; 59: 230–241.
  21. Sawaguchi T, Goldman Rakic PS. The role of D1 dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed response task. *J Neurophysiol* 1994; 71: 515–528.
  22. Sohn YH, Kim GW, Huh K, Kim JS. Dopaminergic influences on the P300 abnormality in Parkinson's disease. *J Neurol Sci* 1998 Jun 11; 158(1): 83–87.