

ORIGINAL ARTICLE

Traffic Noise: A Silent Killer of Male Gamete of Albino Rats**Purushottam Pramanik* and Snehangshu Biswas***Department of Physiology, Hooghly Mohsin College, Chinsurah, Hooghly-712101
West Bengal, India*

Abstract: *Objectives:* The study aims to estimate the change in the weight of testis, sperm count, sperm morphology, testicular cholesterol and protein level in acute and chronic traffic noise exposed albino rats. *Background:* Road traffic is a significant source of noise pollution, a type of environmental stress. Traffic noise significantly increases adrenal cortical function and causes testicular dysfunction. *Method:* Experiment was performed on adult male albino rats. Animals were divided in to one control group and two experimental groups. Both the experimental groups were exposed to traffic noise having intensity 80 dB to 90 dB at the rate of 5 hr daily and control group was not exposed. First experimental group was exposed to traffic noise for 30 days and second group for 60 days. Testicular weight, sperm count, sperm morphology, testicular protein level and testicular cholesterol level were estimated. Statistical analysis was done using unpaired 't' test. *Result:* Contribution of testis to body weight, testicular protein level and sperm count were low but testicular cholesterol level was more in traffic noise exposed rats than unexposed counterpart. Traffic noise exposure also increased percentage of morphologically abnormal sperm. *Conclusion:* Traffic noise adversely affect male gamete and such effect may be due to noise-induced suppression of testosterone synthesis.

Key words: Traffic noise, sperm count, sperm morphology, male infertility.

Introduction

Noise is the most prevalent and insidious natural pollutants of all environmental. Noise exposure not only hampers auditory efficiency but also causes non-auditory physical health effect include change in blood, heart rate and level of stress hormones. The long term effects of noise exposure are well documented as being as chronic exposure of rats to textile industrial noise triggers cytological changes in the adrenal that suggests the existence of a sustained stress response. Exposure of rats to 85 dB noise significantly decreases testicular function and increases adreno cortical [1]. Occupational noise is now considered as a model of chronic stress [2]. Most of the studies supports noise as stress as noise exposure associated with alteration of adrenal adrenocorticosterone and noradrenalin hormone which are primarily considered as stress hormones [3]. Testosterone production was adversely affected by noise stress [4]. Glucocorticoid-induced suppression of testosterone biosynthesis was also seen in male mice exposed to immobilization stress [5]. Chronic immobilization stress provoked an increase in serum corticosterone which caused the decline in testosterone concentration [6]. Road traffic is the most important source of community noise. Even though very high level of traffic noise i.e. average day-night weighted equivalent noise level exceeding 65 dB seem to be stabilized in some countries, the group living in dwellings exposed to 55 to 65 dB is increasing [7].

A review report in 2006 [8] indicates that exposure to 60 dB noise have no adverse health effect. A recent meta analysis indicates that noise exposure above 60 dB increases risk of cardiovascular effects [9]. A number of recent studies have provided further evidence for association between traffic noise and ischemic heart disease [10] and road traffic noise and hypertension [11, 12]. Road traffic noise is a great problem in our country particularly to the people who live beside the road or near the high way. Therefore the main objective of the present study is to investigate effect of traffic noise stress on male gametes.

Material and Methods

Animals and grouping: Experiments were carried out on pure wister strain male albino rats having body weight 140 gm to 180 gm. The animals were housed in standard laboratory condition in a photoperiod cycle of 12 hr: 12 hr (light and dark) and were supplied with standard laboratory diet and drinking water *ad libitum*. Animals were divided in to three groups—one control group and two experimental groups. Each group having 10 animals.

Noise collection and treatment: At first traffic noise was measured by sound level meter from a busiest area near the Khadinamore, Chinsurah , Hooghly, west Bengal. Noise intensities were measured at 10 a.m and 6 Pam (busiest time of the day) for 7 days. Traffic noise was then recorded by a recorder from the same place. Average intensity of sound is then calculated (fig.1). Both the experimental groups were exposed to traffic noise having intensities of 80 dB to 90 dB at the rate of 5 hr daily. Experimental group of animals were kept in a sound proof room for five hours daily for noise exposure. The said animal groups were again transferred to normal laboratory condition with control group for the rest period of time. The treatment has been done for 30 days in acute group and 60 days in chronic group of animals. Control group of animals were maintained in normal area providing the same laboratory condition to all the three groups.

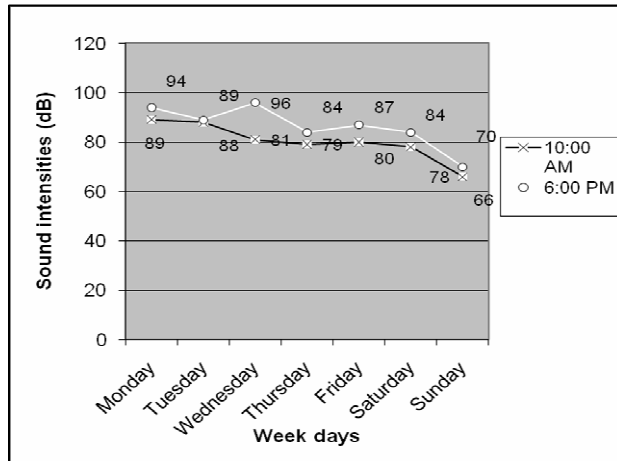


Figure-1: Sound intensities of noise on different week days easured at 10 am and 6 pm near the Khadinamore, Chinsurah, Hooghly.

Measurement of body weight and testis weight: Body weight of each animal was measured after the treatment is over. Animals were killed by decapitation. Testis and epididymis were then dissected out, freed from adhesive fat and connective tissues. Weight of testis and epididymis were measured. Weight of testis was expressed as percentage of body weight.

Separation of epididymal sperm: Epididymal spermatozoa were separated by modification of method of Brooks, 1976 [13]. 2 cm part from caudal portion of epididymis was cut out. It was then cut in to small pieces by sharp blade. Spermatozoa from epididymal pieces were removed by vortexing gently in Krebs Ringer phosphate buffer (pH 7.4). Suspension was used for sperm count.

Sperm count: Spermatozoa were counted as per the method of Zaneveld and Polakoski, 1977 [14]. Sperm suspensions were placed on both side of Neubauer's hemocytometer and allow to settle for 15 min. The number of spermatozoa in the appropriate squares of the hemocytometer was counted under the microscope at 100x magnification.

Sperm morphology: For the study of sperm morphology Spermatozoa were expressed out by cutting the distal end of cauda of Epididymal tubule. Spermatozoa with Epididymal fluid were diluted with physiological saline and a smear was prepared. It was then fixed with methyl alcohol and stained with basic Fuchsin. The slide was then examined under oil immersion objective. Total 200 sperm were counted irrespective to morphology at first out of which number of morphologically abnormal sperms were noted [15]. Result was expressed as percentage on the basis of total sperm count.

Biochemical estimation of testicular protein: Testis was homogenized in 0.9% sodium chloride solution (1 ml per 50 mg tissue). Suspension was allow to settle for 5 min. Supernatant was centrifuged at 800 x g for 15 min [16]. Protein was estimated by Biuret method [17]. The result was expressed as mg / gm weight of testis.

Biochemical estimation of testicular cholesterol: Testis was homogenized in ether-alcohol mixture (1 ml per 50 mg tissue). Suspension was allow to settle for 5 min. Supernatant was centrifuged at 3000 rpm for 10 min. Estimation of cholesterol is done by ferric chloride method [18].

Statistical analysis : Results were expressed as mean \pm standard error of mean. Significance was determined by students' t test. Differences were considered significant when $p < 0.05$.

Results

Effect of traffic noise on testicular weight: Exposure to traffic noise decreases testicular weight. Contribution testis to total body weight is less in experimental groups than control group(fig. 2). The result is more significant in chronic experimental group than acute experimental group.

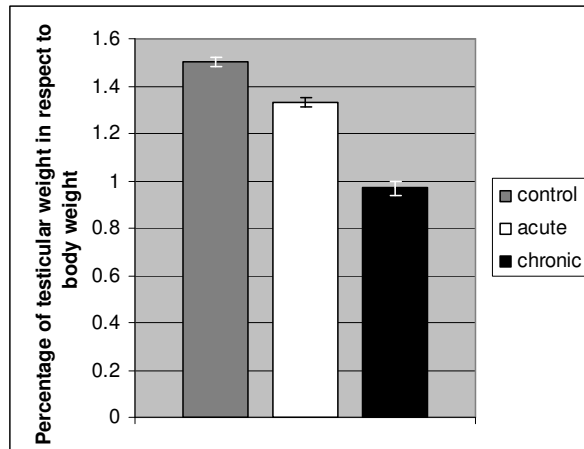


Figure-2: Simple bar diagram showing the result of effect of traffic noise on testis weight (% of total body weight) with the control group of albino rat. Bars represent mean value (n=6) and vertical line as SEM (p < 0.001).

Effect on sperm count: Acute and chronic exposure to traffic noise causes significant decrease of sperm count. The result is more significant when sperm count is expressed after considering both the length of epididymis and the weight of testis than only consideration of the length of epididymis (fig.3).

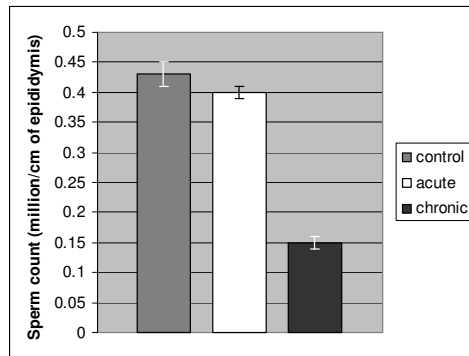


Figure-3 (a): simple bar diagram showing the result of effect of traffic noise on sperm count (number of sperm/cm length of epididymis) albino rats. Bars represent mean value(n=6) and vertical line as SEM (p < 0.05 for acute experiment, p < 0.001 for chronic experiment).

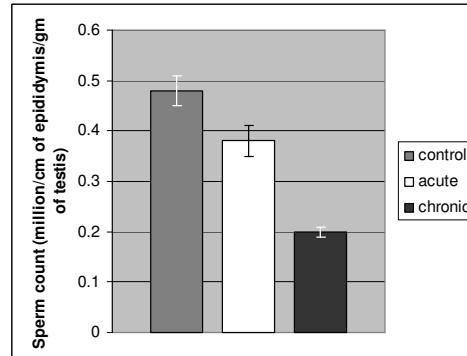


Figure-3 (b): Simple bar diagram showing the result of effect of traffic noise on sperm count (number of sperm/cm length of epididymis/gm of testis) albino rats. Bars represent mean value (n=6) and vertical line as SEM (p < 0.05 for acute experiment, p<0.001 for chronic experiment).

Effect of noise on sperm morphology: Exposure to traffic noise causes a significant change of sperm morphology. The result is more significant in chronic experimental group than acute experimental group. Number of morphologically abnormal sperm in experimental groups of rats is significantly more than control group of rats (fig. 4).

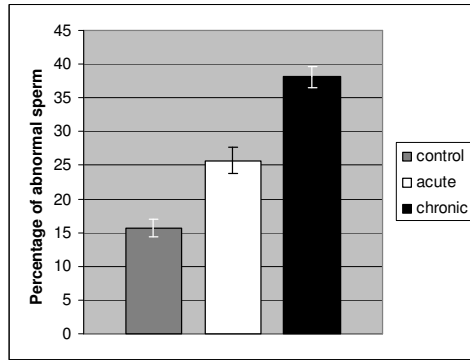
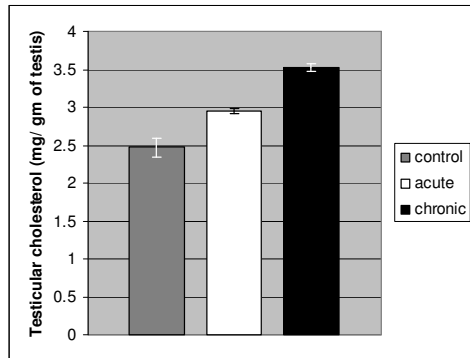


Figure-4: Simple bar diagram showing the result of effect of traffic noise on sperm morphology (% of abnormal sperm in epididymis) of albino rats.

Bars represent mean value (n=6) and vertical line as SEM (p< 0.001).



Effect of noise on protein level of testis: Fig. 5 shows the effect of traffic noise exposure on testicular protein level. Both acute and chronic exposure to traffic noise decreases testicular protein level. The effect is more significant in chronic experimental group than acute experimental group.

Figure-5: Simple bar diagram showing the result of effect of traffic noise on testis cholesterol (mg/gm of testis) with the control group of albino rats.

Bars represent mean value (n=6) and vertical line as SEM (p< 0.05 for acute experiment, p<0.001 for chronic experiment).

Effect of noise on testicular cholesterol level: Exposure to traffic noise significantly increases cholesterol level in testis (fig. 6). The effect is more pronounced in chronic group than acute group.

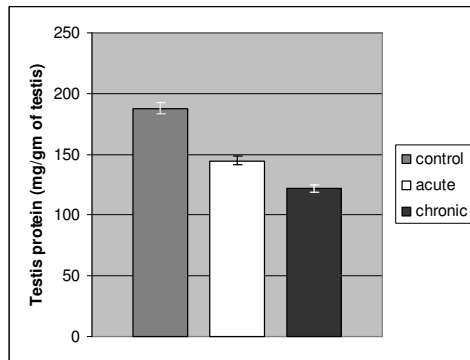


Figure-6: Simple bar diagram showing the result of effect of traffic noise on testis protein (mg/gm of testis) of albino rats.

Bars represent mean value (n=6) and vertical line as SEM (p<0.0001).

Discussion

Results of the study indicate that both acute and chronic exposure to traffic noise having intensity 80 dB to 90 dB have an adverse effect on Testicular weight (Fig.2, sperm count (Fig.3) and sperm morphology (Fig.4)of male albino rats. This effect may be due to disturbance of normal regulation of spermatogenesis.

Gonadotrophic hormone, LH and FSH are important regulators of spermatogenesis. LH enhances the transport of cholesterol into mitochondria from cytosol in interstitial cell of Leydig [19]. This cholesterol is precursor of androgen, testosterone. The cholesterol needed for testosterone biosynthesis can be imported into cell from circulating cholesterol or it can be synthesized within cell either de novo from acetate or from cholesterol ester [20]. On the other hand FSH stimulates production of androgen binding protein (ABP) in Sertoli cell. ABP concentrates testosterone in seminiferous tubule of testis. FSH and testosterone synergistically act in germ cells maturation [21]. However testosterone alone can induce germ cell maturation independent of FSH activity [22].

There is increased corticosterone release following chronic exposure of rat to noise [23]. ACTH secretion increases during stress condition that activates the hypothalamo-pituitary-adrenal axis [24]. It is also suggested that during stress glucocorticoids secretion increases and consequently circulating testosterone level is decreased via glucocorticoid receptor in Leydig cell [25]. In noise-exposed rats plasma testosterone levels were significantly reduced [26, 1]. Exposure to traffic noise in our experiment significantly increases testicular cholesterol level (Fig. 6). This finding suggests that noise exposure decreases testosterone biosynthesis and causes accumulation of cholesterol in testis.

Testosterone acts primarily at stages VII and VIII of spermatogenesis as degeneration of germ cell is first apparent at stages VII and VIII after testosterone depletion [27]. Testosterone stimulates synthesis of specific protein by Sertoli cell in culture [28]. Several androgen-regulated proteins are secreted predominantly by seminiferous tubules at stages VI to VIII of spermatogenic cycle [29]. Secretion of androgen-regulated proteins is reduced due to destruction of Leydig cells by ethane dimethane sulfonate (EDS) but it could be maintained by administration of testosterone. Recent evidences indicate that numbers of androgen-regulated proteins are derived from germ cells [30]. Exposure to traffic noise significantly decreases testicular protein level (Fig. 5). This observation suggests that adverse effect of traffic noise on testicular weight, sperm count and sperm morphology may be due to decrease of testicular protein from noise-induced suppression of testosterone synthesis.

References

1. Ghosh D, Ghosh S, Chattopadhyay S and Debnath J. Effect of noise exposure (85 dB) on testicular adrenocortical steroidogenic key enzymes, acid and alkaline phosphatase activities of sex organs in mature albino rats. *J Environ Sci* 2000;12: 286-289.
2. Nawrot PS, Cook RO and Hamm CW. Embryotoxicity of broadband and high frequency noise in the CD-1 mouse. *J toxicol Environ health* 1981; 8:151-157.
3. Prabhakaran K, Suthanthirarajan N and Namasivayam A. A biochemical changes in acute noise stress in rats. *Indian J Physiol Pharmacol* 1988; 32:100-104.
4. Armario A and Castellanos JM. Effects of noise stress on testosterone secretion in mice. *IRCS. Med Sci* 1984; 12:208-210.

5. Dong Q, Salva A, Sottas CM, Niu E, Holmes M and Hardy MP. Rapid glucocorticoid mediation of suppressed testosterone biosynthesis in male mice subjected to immobilization stress. *J Androl* 2004; 25:973-981.
6. Swami C, Ramanathan J and Jeganath C. Noise exposure effect on testicular histology, morphology and male steroidogenic hormone. *Malaysian J Med Sci* 2007; 14:28-35.
7. Bodin T, Albin M, Ardo J, Stroh E, Ostergren PO and Bjork J. Road traffic noise and hypertension: results from cross sectional public health survey in southern Sweden. *Environmental Health* 2009; 8:dor 10.1186 /1476-069x-8-38.
8. Babisch W. Transportation noise and cardiovascular risk: updated review and synthesis of epidemiological studies indicate that the evidence has increased. *Noise Health* 2006; 8(30):1-29.
9. Babisch W. Road traffic noise and cardiovascular risk. *Noise Health* 2008; 10(30):27-33.
10. Selander J, Nilsson ME, Bluhm G, Rosenlund M, Lindquist M, Nise G and Pershagen G. Long term exposure to road traffic noise and myocardial infraction. *Epidemiology* 2009; 20(2):272-279.
11. Barregard L, Bonde E and Ohrstrom E. Risk of hypertension from exposure to road traffic noise in a population based sample. *Occup Environ Med* 2009; 66(6):410-415.
12. Leon bluhm G, Berglind N, Nordling E and Rosenlund M. Road traffic noise and hypertension. *Occup Environ Med* 2007; 64(2):122-126.
13. Brooks DE. Activity of androgenic control of glycolytic enzymes in the epididymis and Epididymal spermatozoa of the rat. *Biochem J* 1976; 156:527-537.
14. Zaneveld LJD and Polakoski KL. Collection and physical examination of the ejaculate, in Techniques of Human Andrology ed. Hafez ESE, *North Holand Biomedical Press, Amsterdam* 1977;147-156.
15. Mukherjee KL. Semen analysis in Medical Laboratory Technology. *Academic publishers*, 1988; 871- 879.
16. Srikanth V, Malini T, Arunakaran J, Govindarajulu P and Balasubramanian K. Effect of ethanol treatment on Epididymal secretory products and sperm maturation in albino rats. *J Pharmacol Expt Therap* 1999; 288 (2): 509-515.
17. Mc Murray JR, Plasma Protein. In Gowenlock AH, editor. Varley's Practical Clinical Biochemistry, 6th Ed, *CBS publishers (Indian Reprint), New Delhi*, 2002; 407-408.
18. Nath RL and Nath RK. Practical Biochemistry in clinical Medicine. *Academic Publisher* 1990; 114.
19. Barlow NJ, Philips SL, Wallace DG, Sar M, Gaido KW and Foster PM. Quantitative changes in gene expression in the fetal rat testis following exposure to di (n-butyl) phthalate. *Toxicol Sci* 2003; 73: 431-441.
20. Cao G, Zhao L, Stangl H, Hasegawa T, Richardson JA, Parker KL and Hobbs HH. Development and hormonal regulation of murine scavenger receptor, class B, Type 1. *Mol Endocrinol* 1999; 13:1460-1473.
21. Haywood M, Apaliviero J, Jimenez M , King NJ, handelsman DJ and Allan CM. Sertoli and germ cell development in hypogonadal mice expressing transgenic FSH alone or in combination with testosterone. *Endocrinology* 2003; 144:509-517.
22. Spaliviero JA, Jimenez M, Allan CM and Handelsman DJ. LH receptor mediated effects on inhibition of spermatogenesis in gonadal deficiency mice are replicated by testosterone. *Biol Reprduc* 2004; 70:32-38.
23. Gesi M, Fornae F, Lenzi P, Natale G, Soldani P and Paparelli A. Time dependent changes in adrenal cortex ultrastructure and corticosterone level after noise exposure in male rats. *Eur J Morphol* 2001; 39:129-135.

24. Pignatelli D, Magalhaes MM and Magalhaes MC. Direct effect of stress on adrenal cortical function. *Horm Metab Res.* 1998; 30: 464-474.
25. Pelligrini A, Soldoni P, Gesi M, Lenzi P and Martin F. Diazepam reduced ultrastructural changes induced by noise stress in rats adrenal gland. *J Submicro Cytol Pathol* 1998; 30:385-39.
26. Orr TE, Taylor MF, Bhattacharyya AK, Collins DC and Mann DR. Acute immobilization stress disrupts testicular steroidogenesis in adult male rats by inhibiting the activities of 17 alfa hydroxylase and 17, 20-lyase with out affecting the binding of LH /hCG receptors. *Androl* 1994; 15:302-308.
27. Russel LD and Clerment Y. Degeneration of germ cells in normal, hypophysectomised and hormone treated hypophysectomised rats. *Anal Rec* 1977; 187:347-366.
28. Robert K and Griswold MD. Testosterone induction of cellular protein in culture sertoli cells from hypophysectomised rats and rats of different ages. *Endocrinology* 1989; 125:1174-1179.
29. Sharpe RM, Moddocks S, Millar M, Kerr JB, Saunders PTK and McKinnell C. Testosterone and spermatogenesis: identification of stage specific androgen regulated proteinsecreted by adult rat seminiferous tubules. *J Androl* 1992; 13:172-184.
30. Sharpe RM, Millar M and McKinnell C. Relative roles of testosterone and germ cell complement in determining stage-dependent changes in protein secretion by isolated rat seminiferous tubules. *Int J Androl* 1993; 16:71-81.

*All correspondences to: Dr. Purushottam Pramanik, Post Graduate Department of Physiology, Hooghly Mohsin College, Chinsurah, Hooghly-712101 West Bengal, India. Email: puru.pra@gmail.com