

NEUROIMMUNO MODULATION BY VESTIBULAR STIMULATION IN COLD WATER SWIMMING STRESS INDUCED WISTAR ALBINO RATS

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ABSTRACT

Objectives: We hypothesized that vestibular stimulation prevents stress-induced suppression of immunity by inhibiting the stress axes and to test this hypothesis total leucocyte count, differential leucocyte count, platelet count, hemoglobin level, organ weight of spleen and liver were observed following vestibular stimulation in cold water swimming stress induced Wistar albino rats.

Methods: Totally, 24 healthy, adult male albino rats of Wistar strain were used in the present study. Rats were forced to swim in the plastic tubs (height: 60 cm, diameter: 40 cm) containing cold water, maintained at 10°C. The middle ear cavity was irrigated with hot (40°C) or cold (15°C) water through a polyethylene tube for 15 days.

Results: Data were analyzed by SPSS 20.0. Statistical tests used are two-way RM ANOVA and Bonferroni post-tests. Cold water vestibular stimulation was significantly prevented effects of stress on hematological parameters ($p < 0.05$). Both cold and hot water vestibular stimulation effectively controlled stress induced changes on body weight and organ weights ($p < 0.05$).

Conclusion: Maximum effect of stress was observed on the 7th day in stress only group whereas this effect is minimized in cold water vestibular stimulation group and maintained in normal limits thereafter. We recommend further detailed study in this area is considered for further detailed study.

Keywords: Caloric vestibular stimulation, Cold water swimming stress, Immunity.

INTRODUCTION

Protective immunity is conferred by the complementary actions of two separate but interdependent components of the immune system: The innate immune system and the adaptive or acquired immune system. The responses of these two systems differ in timing and in the selectivity of the defense mechanisms [1]. The innate immune system encompasses the body's non-specific immune responses that come into play immediately on exposure to a threatening agent. In contrast, the adaptive or acquired immune system relies on specific immune responses selectively targeted against a particular foreign material to which the body has already been exposed.

Vestibular apparatus is located in the inner ear and it is the sense organ for balance. It starts functioning from the 5th month of gestation [2]. One of the newest and most popular therapies for developmentally delayed children is vestibular stimulation. Controlled vestibular stimulation by swing can be applied not only as an intervention for learning disability but also to relieve stress, cancer pain, to promote sleep to improve immunity and also to treat endocrine disorders [3-7].

Stress may be defined as lack of congruence between important aspects of the person and his perceived environment [8]. Stress is a priceless poison of 21st century; stress harms different parts of the human body from muscles and tissues to organs and blood vessels. It increases pulse rate, respiration, blood pressure and body temperature. It can also interfere with the body metabolism, digestion, appetite, sleep, sexuality, and fertility [9]. Stress will increase susceptibility to infections by suppressing immunity. However, the effect of acute and chronic stress on immune functions varies. Acute stress cellular immunity whereas chronic stress suppresses both cellular and humoral immunity [6]. It was reported that noise stress significantly decreases total leucocyte count (TLC) in rats [10] however, chronic exposure to noise stress affects neutrophil functions while corticosterone and total leukocyte count gets adapted [11].

We hypothesized that vestibular stimulation prevents stress-induced suppression of immunity by inhibiting the stress axes. To test this hypothesis total leucocyte count, differential leucocyte count, platelet count, hemoglobin level, organ weight of spleen and liver were observed following vestibular stimulation in cold water swimming stress induced Wistar albino rats.

METHODS

The present study was approved by Institutional Ethical Committee no: EC/4/23/5/24.

Animals

Totally, 24 healthy, adult male albino rats of Wistar strain were used in the present study. Rats were housed under standard laboratory conditions with food and water provided *ad libitum*. Rats were randomly assigned into four groups.

Group A: (n=6) Control group (neither stress nor vestibular stimulation).

Group B: (n=6) Stress group (cold water swimming stress only).

Group C: (n=6) Cold water swimming stress + vestibular stimulation with cold water applied for 15 days.

Group D: (n=6) Cold water swimming stress+ vestibular stimulation with hot water applied for 15 days.

Cold water swimming stress

Rats were forced to swim in the plastic tubs (height: 60 cm, diameter: 40 cm) containing cold water, maintained at 10°C. Depth of the water in the plastic tub was 30 cm. The swimming session lasted for 45 minutes daily. Experiments were done between 9 am and 12 noon to minimize the circadian variability [14].

Caloric vestibular stimulation

The middle ear cavity was irrigated with hot (40°C) or cold (15°C) (Fig. 1) water through a polyethylene tube for 15 days [15,16].

Body weight was measured before and after the stress period in all the rats. The animals were sacrificed by decapitation and blood samples were collected. The wet weight of the organs (liver, spleen) was expressed per 100 g of body weight. TLC, DLC, platelet count, and hemoglobin were estimated by standard methods.

Data analysis

Data were analyzed by SPSS 20.0. Statistical tests used are two-way RM ANOVA and Bonferroni post-tests.

RESULTS

The results are presented in Figs. 2-8.

Hemoglobin levels increased significantly on 1st day in stress + hot water vestibular stimulation group, on 7th day in stress only, stress+ cold water vestibular stimulation groups, on 15th day in stress only, stress + cold water vestibular stimulation, and stress + hot water vestibular stimulation when compared to control group. Hemoglobin

levels decreased significantly on the 7th day in hot water vestibular stimulation group when compared to stress only group, however, it is not statistically significant when compared to control group.

TLC is decreased in the stress only group when compared to control group. However, it is not statistically significant. TLC increased in stress + cold water vestibular stimulation and stress+ hot water vestibular stimulation when compared to control group. A significant increase in TLC is observed in stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups when compared to stress only group on 7th day. On 15th day, TLC increased in stress + cold water vestibular stimulation group and decreased in stress + hot water vestibular stimulation group. However, it is not statistically significant. The difference between stress + cold water vestibular stimulation and stress + hot water vestibular stimulation is statistically significant.

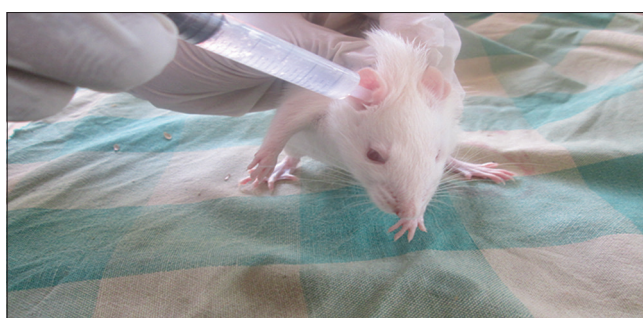


Fig. 1: Method of caloric vestibular stimulation

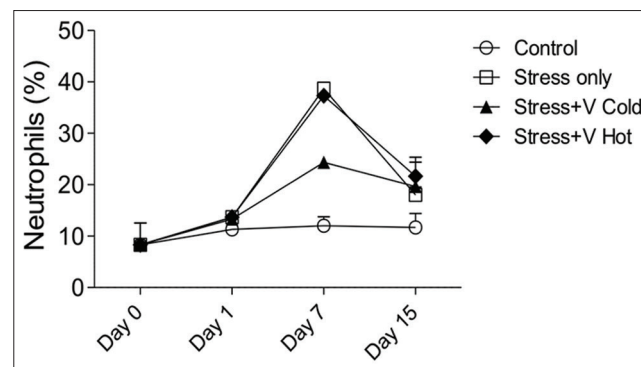


Fig. 4: Neutrophils count in control, stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups

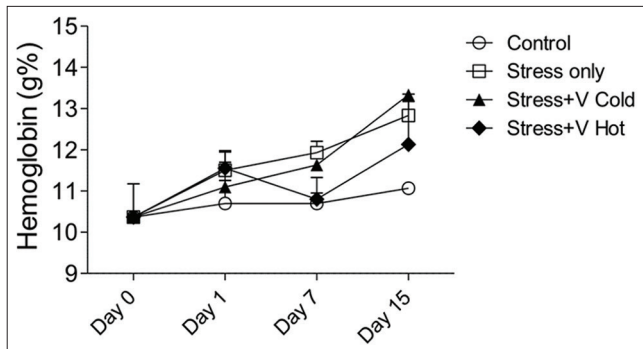


Fig. 2: Hemoglobin levels in control, stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups

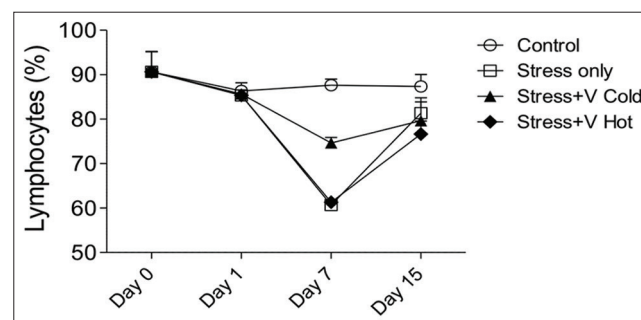


Fig. 5: Lymphocytes count in control, stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups

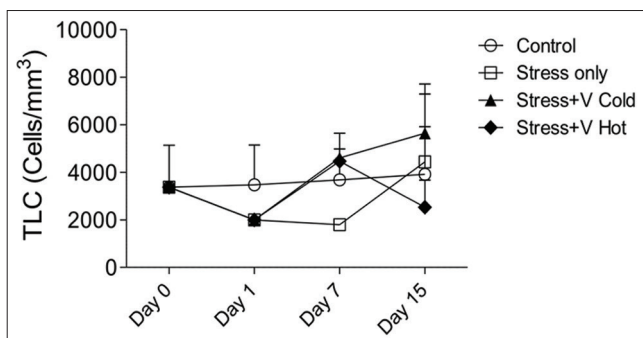


Fig. 3: Total leucocyte count in control, stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups

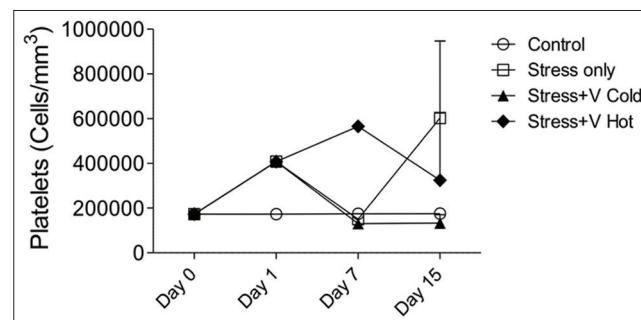


Fig. 6: Platelets count in control, stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups

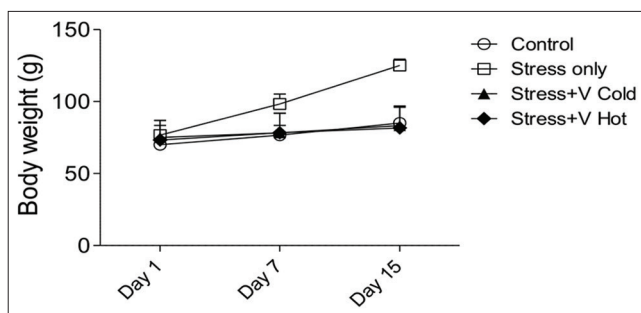


Fig. 7: Body weight in control, stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups

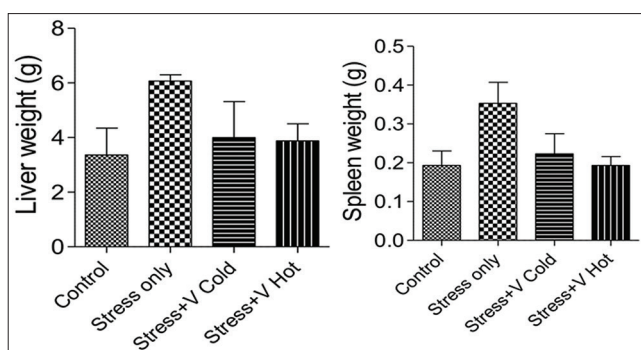


Fig. 8: Liver and spleen weight in control, stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups

Neutrophil count increased significantly on 7th, 15th day in stress only, stress+ cold water vestibular stimulation and stress+ hot water vestibular stimulation groups. This increase in the neutrophil count is statistically significant when compared with stress + cold water vestibular stimulation.

Lymphocyte count decreased significantly on 7th, 15th day in stress only, stress + cold water vestibular stimulation, and stress + hot water vestibular stimulation. Decrease is statistically significant in the control group when compared with stress only group and stress + cold water vestibular stimulation group.

Platelet count increased significantly on 1st day in stress only, stress + cold water vestibular stimulation and stress + hot water vestibular stimulation group when compared with control group. Platelet count increased significantly in stress + hot water vestibular stimulation group when compared with control group. Platelet count increased significantly on 15th day in stress only group, when compared with control group. Platelet count decreased significantly on 15th day in stress + cold water vestibular stimulation group when compared with stress only group. This change is not statistically significant with control group. Platelet count increased significantly in stress + hot water vestibular stimulation group on 7th day when compared with control group, stress only group and stress + cold water vestibular stimulation groups. In stress + hot water vestibular stimulation group, platelet count decreased on 15th day when compared with stress only group, but increased significantly when compared with stress + cold water vestibular stimulation group.

Body weight increased significantly in stress only group when compared with the control group on 7th and 15th day. Body weight decreased significantly in stress+ cold water vestibular stimulation and stress+ hot water vestibular stimulation groups when compared with stress only group.

Liver weight increased significantly on 15th day in stress only group when compared with control group. Liver weight decreased significantly in stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups when compared with stress only group.

Spleen weight increased significantly on 15th day in stress only group when compared with control group. Spleen weight decreased significantly in stress + cold water vestibular stimulation and stress + hot water vestibular stimulation groups when compared with stress only group.

DISCUSSION

It was reported that hemoglobin levels increases significantly followed by cold water stress [17-19]. In the present study, we have observed a significant increase in hemoglobin levels in stress only group on 7th and 15th day. However, this increase is prevented in stress + hot water vestibular stimulation group. This effect may be due to inhibition of the effect of cortisol on the production of RBC [4].

We agree with the previous studies as we have observed a decrease in the TLC in stress only group on 7th day [20,21]. However, this decrease is not observed in both stress+ cold and hot water vestibular stimulation groups. This effect might be due to vestibular induced changes in adrenal steroids. Long-term applications of effects of hot and cold vestibular stimulation on TLC are different.

Neutrophil count increased significantly in stress only group is in accordance with previous studies [22]. However, in stress + cold water vestibular stimulation Neutrophil count is significantly less than stress only group.

Lymphocyte count decreased significantly in stress only group on 7th day is in accordance with previous studies [4]. This decrease might be due to inhibition of lymphocyte mitotic activity by glucocorticoids. This effect of stress on lymphocyte is minimum in stress + cold water vestibular stimulation group.

Glucocorticoids increases number of platelets in the circulation [4]. We agree with the previous studies as we have observed a significant increase in platelets on 15th day. However, platelet count was within normal limits in stress + cold water vestibular stimulation group.

It was reported that body weight increased significantly after 7 days of cold water swimming stress and no change after 15 days [14]. In the present study, significant increase in the body weight observed after 7 days and 15 days of cold water swimming stress in stress only group and body weight remain in normal limits in both hot and cold water vestibular stimulation groups. This effect may be due to the vestibular balance of food intake [2].

It was reported that liver and spleen weight increased significantly followed by cold water swimming stress [19]. We agree with the previous studies as we have observed a significant increase in the liver and spleen weight. No significant changes were observed in stress + cold and hot water vestibular stimulation groups.

CONCLUSION

Maximum effect of stress was observed on 7th day in stress only group whereas this effect is minimized in cold water vestibular stimulation group and maintained in normal limits thereafter. We recommend further detailed study in this area.

is considered for further detailed study.

REFERENCES

1. Sherwood L. Essentials of Physiology. 4th ed. Delhi: Brooks/Cole, a Part of Cengage Learning; 2013. p. 332-3.
2. Sailesh KS. Vestibular balance of food intake. Int J Pharm Bio Sci

- 2014;5(3):(B)1069-73.
3. Sailesh KS, Archana R, Antony NJ, Mukkadan JK. You are never too old to swing. *Res J Pharm Biol Chem Sci* 2014;5(5):612-5.
 4. Sailesh KS, Archana R, Mukkadan JK. Controlled vestibular stimulation: A physiological method of stress relief. *J Clin Diagn Res* 2014;8(12):BM01-2.
 5. Sailesh KS, Archana R, Mukkadan JK. Thinking with your sixth sense. *Res J Pharm Biol Chem Sci* 2014;5(4):481-5.
 6. Sailesh KS, Mukkadan JK. Psychoneuroimmuno modulation by controlled vestibular stimulation. *J Clin Exp Res* 2013;1(3):68-70.
 7. Sailesh KS, Archana R, Antony NJ, Mukkadan JK. Controlled vestibular stimulation: Supplementary treatment for hypothyroidism. *Res J Pharm Biol Chem Sci* 2014;5(3):1842-5.
 8. Mythri H, Saxena S, Ananda SR, Chandu GN. Perceived sources of stress among bioallied science students of south India. *Int J Pharm Pharm Sci* 2014;6(8):335-9.
 9. Balaji Deekshitulu PV. Vedic life style in stress control. *Innovare J Health Sci* 2014;2(2):1-3.
 10. Archana R, Namasivayam A. The effect of acute noise stress on neutrophil functions. *Indian J Physiol Pharmacol* 1999;43(4):491-5.
 11. Archana R. Effect of chronic noise stress on neutrophil functions in rats. *Am Int J Res Form Appl Natl Sci* 2013;3(1):88-92.
 12. Sailesh KS, Mukkadan JK. Can controlled vestibular stimulation reduce stress? *Health Sci* 2013;2(3):(JS001)1-13.
 13. Winter L, Kruger TH, Laurens J, Engler H, Schedlowski M, Straumann D, et al. Vestibular stimulation on a motion-simulator impacts on mood States. *Front Psychol* 2012;3:499.
 14. Nagaraja HS, Jaganathan PS. Forced swimming stress-induced changes in the physiological and biochemical parameters in albino rats. *Indian J Physiol Pharmacol* 1999;43(1):53-9.
 15. Nishiike S, Nakamura S, Arakawa S, Takeda N, Kubo T. GABAergic inhibitory response of locus coeruleus neurons to caloric vestibular stimulation in rats. *Brain Res* 1996;712(1):84-94.
 16. Miller SM, Ngo TT. Studies of caloric vestibular stimulation: Implications for the cognitive neurosciences, the clinical neurosciences and neurophilosophy. *Acta Neuropsychiatr* 2007;19:183-203.
 17. Ali KS. Impact of cold stress on haematological and biochemical parameters of yemeni toad (*Bufo tihamicus*). *Res J Pharm Biol Chem Sci* 2013;4(3):1059.
 18. Aarif O, Mahapatra PS. The effect of cold stress on biochemical and hematological parameters in broad breasted white turkeys. *Wyno J Biol Sci* 2013;1(4):20-3.
 19. Krishnan J, Sailesh KS, Archana R, Mukkadan JK. Effect of forced freshwater and cold water swimming stress induced changes in selected physiological and biochemical parameters in wistar albino rats. *Res J Pharm Biol Chem Sci* 2015; 6(3):750-54.
 20. Dhabhar FS, Miller AH, McEwen BS, Spencer RL. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *J Immunol* 1995;154(10):5511-27.
 21. Joseph IM, Suthanthirarajan N, Namasivayam A. Effect of acute heat stress on certain immunological parameters in albino rats. *Indian J Physiol Pharmacol* 1991;35(4):269-71.
 22. Sembulingam K, Sembulingam P, Namasivayam A. Effect of acute noise stress on some selected stress indices. *Biomedicine* 1996;16(1):23-6.