Effect of vestibular stimulation in cold water stress induced neurological changes

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ABSTRACT
The current study was undertaken to evaluate the effects of cold water stress on the brain and to evaluate the beneficial effect of vestibular stimulation on stress-induced brain changes. Healthy, male, Wistar rats, weighing 180 to 250 gm with 3-6 months of age, were used for the study. Stress was induced by making the animals swim in cold water maintained at 10°C for 30 min a day, for 7 days. Following cold water swimming stress, bilateral hot water caloric vestibular stimulation was given to the animals using 41°C temperature water for 15 days. At the end of the experiment, serum corticosterone was measured. Rats were sacrificed and histopathological brain changes were studied by Hematoxylin & Eosin staining. Serum corticosterone level has increased significantly after cold water swimming stress (p<0.01). Corticosterone was less in animals that received caloric vestibular stimulation in comparison with the animals which did not receive caloric vestibular stimulation (p<0.05). Coldwater swimming stress had induced neuronal atrophy with congested blood vessels and mononuclear cell inflammatory infiltrate in Hippocampus. Hypothalamus showed Focal neuronal atrophy with congested blood vessels. Stressed animals that received caloric vestibular stimulation recovered well and showed the cerebral cortex with the normal
neuroglial arrangement. Hypothalamus showed normal morphology and the hippocampus showed a pyramidal layer with a normal thickness in comparison to the animals which did not receive caloric vestibular stimulation. We conclude Caloric vestibular stimulation was effective in reversing the cold water stress-induced serum corticosterone and histopathological changes in the brain.

Key words: Stress, Cold water swimming stress, Caloric vestibular stimulation, Corticosterone, Histopathological changes

INTRODUCTION

The name “stress” was first used by Hans Selye, founder of the stress theory. Any alteration in the physiological balance is stress. The reaction to stressor varies largely between individuals and the stress cycle is comprised of four phases: the resting ground phase, the tension phase, the response phase and the relief phase (Rom and Reznick, 2016). One of the important systems responding to stress is the activation of the hypothalamic-pituitary-adrenal (HPA) axis to ensure appropriate response to the stressor. Chronic stress, which is associated with changes in the hippocampus, may be associated with the onset of psychotic disorders (Phillips et al., 2006). Vestibular apparatus is the sense organ for equilibrium and becomes functional from the 5th month of gestation. Traditionally controlled vestibular stimulation was used for neurological diagnosis, but it could be used to investigate and treat other clinical conditions (Miller and Ngo, 2007). Controlled vestibular stimulation has been proven to be helpful in dementia (Jinu et al., 2018), modulation of brain aging neurotransmitters (Sailesh et al., 2014) and in the improvement of depression and anxiety. But very little is known about the effects of vestibular stimulation on stress-induced changes in the brain. The present study was taken up to evaluate the effect of cold water stress on changes in the brain of Wistar rats and to evaluate the effect of caloric vestibular stimulation on stress-induced changes in the brain of Wistar rats.
Animals

Male, Wistar rats of 3-6 months of age, weighing 180 to 350g m were included in the study. Animals were maintained as per the guidelines of the Committee for Control and Supervision of Experiments on Animals. Pellet diet was provided with water ad libitum. Four animals were housed in a polypropylene cage. The present study was carried out after obtaining Institutional animal ethical committee clearance (1/PIMS/2017Dated24/08/2017).

Experimental Design

The animals were randomly selected and grouped as follows:

Group I (n=6) – Control (neither stress nor caloric Vestibular stimulation)

Group II (n=6) – stress for 7 days (cold water swimming stress for 14 days)

Group III (n=6) – stress for 7 days + natural recovery (NR) for 15 days

Group IV (n=6) – stress for 7 days + caloric vestibular stimulation (CVS) for 15 days

Cold Water Swimming Stress

Rats were made to swim in cold water maintained at 10°C for 30 minutes a day between 9.00 AM to 12.00PM. Plastic containers of 60cm height, 40 cm diameter were used and the water level was maintained at 30 cm (Nagaraja and Jeganathan, 1999).

Caloric vestibular stimulation

Caloric vestibular stimulation was given by irrigating external auditory meatus with 2 ml of water maintained at 41°C using a polyethylene tube bilaterally for 15 days (Varghese et al., 2015).

At the end of the experiment, the bloodsample was obtained by retro-orbital puncture and animals were sacrificed by decapitation. Blood was allowed to clot and serum was obtained after centrifugation for 20 min at a speed of 3000 rpm. Serum corticosterone was analyzed using a solid phase enzyme linked immunosorbent assay (ELISA) method. For analysis of histopathological changes brain was placed in 10% neutral buffered formaldehyde. After proper fixation, sections of 3-5 mm were prepared and submitted to dehydration, clearing impregnation and embedding. Sections were made by micro-tome and stained by Hematoxylin and Eosin (H&E).
Statistical Analysis

Data was analyzed using SPSS 20. One-way analysis of variance followed by Tukey’s post hoc test was used for multiple comparisons and expressed as mean ±S.E.M. p-value<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1 explains the changes in body weight in control, stress, stress followed by natural recovery and stress, followed by caloric vestibular stimulation groups. Results are expressed as mean±SEM (n=6). Statistical analysis showed no significant changes between the groups.

As given in Figure 1 shows that, Results are expressed as mean±SEM (n=6). ***p<0.001 as compared to the control group p<0.001 as compared to the stress group. Corticosterone has increased in the stress group (53.22 ± 11.57) when compared to the control group (11.74±0.52). Animals that received caloric vestibular stimulation showed lower levels of corticosterone(49.38 ± 8.85) in comparison to animals which were left for natural recovery (186.98 ± 48.2) after 7 days of stress. The natural recovery group has showed statistically significant increase in serum corticosterone in comparison to both control and stress groups.

Coldwater swimming stress-induced neuronal atrophy with congested blood vessels, nuclear pyknosis and Mononuclear cell inflammatory infiltrate in Hippocampus (Figure 2). Hypothalamus showed Focal neuronal atrophy with congested blood vessels (Figure 3). Stressed animals that received caloric vestibular stimulation recovered well and hypothalamus showed normal morphology and the hippocampus showed a pyramidal layer with a normal thickness in comparison to the animals which did not receive caloric vestibular stimulation.

When Stress is applied for a long duration, it causes hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis (Alkadhi, 2013), which is mediated by the hippocampus (Zhu et al., 2014). The prolonged exposure of the hippocampus to the glucocorticoids disturbs the metabolism of the neurons by inhibiting glucose uptake and makes them more sensitive to metabolic inputs (Sapolsky et al., 1988). Stress inhibits the inhibitory input to the hypothalamic-pituitary-adrenal (HPA) axis (Joëls et al., 2004), resulting in overactivation of the HPA 136 axis, which increases corticosterone.

Brain areas which are targeted by the stress are hippocampus, amygdala and prefrontal cortex (Bremner, 2006). Stress is known to cause morphological rearrangement
(McEwen, 2006), dendritic atrophy in hippocampal pyramidal neurons especially in the CA3, CA4 region and an impairment of neurogenesis in the dentate gyrus (Fuchs et al., 2001; McEwen, 1999; Gill and Grace, 2013), and causes thinning of motor cortex (Khan et al., 2018). Our current study also proves stress induces pathological and morphological changes in the hippocampus and hypothalamus.

The vestibular system has extensive connections with various structures of the brain, which include the hippocampus, amygdala, thalamus, prefrontal cortex (Gurvich et al., 2013). Our previous studies have shown the effectiveness of vestibular stimulation in improving auditory and visual reaction time in stress (Rajagopalan et al., 2017). Caloric vestibular stimulation can inhibit Hypothalamo-Pituitary-Adrenal (HPA) axis and Sympatho-Adrenal-Medullary (SAM) axis by direct pathway and also by increasing the release of GABA and activating hippocampal formation (Kumar et al., 2013) and decreases corticosterone levels.

**CONCLUSION**

Caloric vestibular stimulation is effective in reversing the cold water stress induced corticosterone levels and changes in the brain. The decrease in corticosterone might be the reason for changes in the brain following caloric vestibular stimulation.

**Source of funding** - Self funded

**Conflict of Interest** - Nil.

**REFERENCES**


Table 1: Bodyweight in control, stress, stress followed by natural recovery and stress, followed by vestibular stimulation groups in Wistar rats.
followed by recovery by caloric vestibular stimulation

| BodyWeight (grams) | 287.75 ± 14.32 | 279 ± 23.84 | 318.75 ± 28.17 | 303.33 ± 14.13 |

Results are expressed as mean ±SEM (n=6). p<0.05 considered to be significant.

Figure 1: Serum corticosterone levels in control, stress, stress followed by natural recovery and stress followed by caloric vestibular stimulation induced-Wistar rats
**Figure 2:** Histopathology of hippocampus in Wistar rats under 10x of microscope. a. Control b. neuronal atrophy following stress c. nuclear pyknosis following stress d. 15 days of vestibular stimulation after stress.

**Figure 3:** Histopathology of hypothalamus in Wistar rats under 10x of microscope. a. Control b. neuronal atrophy following stress c. congested following stress d. 15 days of vestibular stimulation after stress.