Role of Caloric Vestibular Stimulation on body weight, glucose levels and renal profile in Chronic Mild Stress-Induced Rats

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Abstract:

Background: Caloric Vestibular Stimulation (CVS) is a neuro-therapeutic technique used for the stimulation of the vestibular system. Stress is referred as a physical, mental, or emotional element that generates physiological or psychological tension. The practice of vestibular stimulation can significantly decrease stress. Hence, the objective of the study was to evaluate the impact of vestibular stimulation on body weight, blood glucose, and renal profile in rats exposed to Chronic Mild Stress (CMS).
Methodology: Four groups (n = 6) of 24 healthy male Sprague Dawley rats were employed. CMS was delivered by various stimuli for 28 days. Hot water (42°C ± 2°C) was used to dispense CVS. Body weight was measured on day 1, 7, 14, 21, 28, 35 and 42. At the end of the study, blood samples were obtained for the analysis of glucose and renal profile.
Results: In CMS-induced rats, CVS treatment preserved body weight and renal function, proving that CVS is advantageous for stress management.
Conclusion: According to the findings of the current study, CVS is a physiological therapy that can be used effectively to relieve stress.

Keywords: Caloric Vestibular Stimulation, Body Weight, Blood Glucose, Renal Profile, Chronic Mild Stress

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Introduction

Stress is defined as the non-specific retort of the body to any unpleasant impetus caused by stress hormones and divergence from homeostasis (Chrousos., 2009). The stimulus, the perception of the stimulus, and the resulting behavioral and physiological responses of the body are all the components of stress (Levine., 2005). The hypothalamic-pituitary- adrenocortical (HPA) axis and the sympathetic-adrenomedullary (SAM) system are typically regarded as the two major contributors to the stress response. Both these key systems can be viewed as a pair of interconnected communication networks designed to synchronize and regulate peripheral physiology at the level of cells, tissues, and organs in accordance with their environment (Koolhaas *et al*., 2011). Chronic Mild Stress (CMS) is a universally used rodent model of depression, that involves repeatedly exposing the animal to a combination of unpredictable and minor stressors over an extended period of time (Hill *et al*., 2012). Since the CMS model has been revealed to cause an anhedonic-like state in rats, which mirrors some stress symptoms in humans, (Bekris *et al*., 2005) it is extensively stared as an representative example and may be utilized to better comprehend abnormal psychology in humans (Willner., 2017).

Caloric vestibular stimulation (CVS) is a universally used therapeutic application for the stimulation of vestibular system which functions to maintain balance, spatial orientation, and processes the visual images (Khan *et al*., 2013). CVS entails transferring either warm or cool temperature to the vestibular organs situated in the surrounding labyrinth, typically *via* water or air. The density of endolymphatic fluid within the semicircular canals and otolith organs changes because of these temperatures, which modifies the firing rates of vestibular hair cells. As the brain interprets the ensuing change in vestibular nerve activity as a natural head movement, several cortical regions experience increased blood flow. (Fasold *et al*., 2002, Dieterichat *et al*., 2003). Walking, dancing, and head movement are all common daily activities that can stimulate the vestibular system (Archana *et al*., 2021). Vestibular stimulation has shown promise in treating several neuropsychiatric disorders. However, the information on how vestibular stimulation affects body weight, blood glucose, and renal function under stress is very scarce. Therefore, the purpose of the current investigation was to ascertain how vestibular stimulation affects the body weight, blood glucose levels, and renal profile of rats given CMS.

Materials and Methods

Experimental animals

Healthy, adult male Sprague Dawley (SD) rats that weighed 180 ± 20 g were utilized. Large, airy, polyacrylic cages were used to house the rats. A 12-hour cycle of light and dark was used to regulate the room's temperature. The rats were given unlimited access to water and regular rat pellet meal. Before using the animals in the experiment, an acclimation period of 5 days was given. The study received approval from the University Human and Animal Ethics Committee (AUAEC/FOM/2020/03) and was carried out in accordance with the standards of the recommendations of the Animal Research Review Panel.

Chronic Mild Stress (CMS) Administration

The CMS model employed in this study is a customized iteration of the CMS method employed in earlier studies (Willner., 2017). Multiple stressors of varying durations were continuously applied for 28 days, one at a time each day, and the cycle was repeated day after day. The rats were subjected to stressors such as restraint stress, overcrowding, wet bedding, forced swimming, tail pinch in restrainer, cold water swim test, inversion of the day and night cycle. Throughout the whole 45-day period, CMS was not administered to the animals in the control group.

Caloric Vestibular Stimulation (CVS)

The middle ear cavity of each rat ear was irrigated using a syringe loaded with 2 mL of warm water (42°C ± 2°C) after setting the rat in a rat restrainer. In order to maintain consistent vestibular stimulation, the flow rate was maintained at 0.1 mL/s. The rats were placed with the horizontal canal tilted 30 degrees from the horizontal plane to generate convection currents in the semicircular canals
(Nishiike *et al*., 1997, Nishiike *et al*., 2000, George *et al*., 2022).

Experimental design

Four groups of six animals each were arbitrarily selected as follows:

Group 1 - Control
Group 2 - CMS
Group 3 - CVS
Group 4 - CMS + CVS

The animals in group 1 were healthy and free of CVS or CMS. The animals in group 2 received CMS with various stressors once daily for 28 days. The rats in group 3 received CVS treatment once daily for 45 days. The rats in group 4 were provoked with both CMS and CVS parallelly starting from day 1, CMS was given for 28 days, and CVS treatment was administered for 45 days. Weight measurements were taken on days 1, 7, 14, 21, 28, 35, and 42. At the end of the study, blood was acquired one day before the animal was euthanized for glucose and renal profile analysis.

Body weight measurement

The body weight of the rats was measured in regular intervals using a mechanical weighing scale.

Blood collection

Under diethyl ether anesthesia, blood was withdrawn from the retro-orbital sinus. In a plain glass tube, 1ml of blood was stored from each animal. For 20 minutes, the blood samples are centrifuged at 3000 RPM. Serum samples were then acquired and used to calculate glucose levels and renal function parameters (Parasuraman *et al*., 2010).

Estimation of blood glucose

The Roche Cobas 8000 fully automatic analyzer was used to quantify blood glucose using the electrochemiluminescence immunoassay technique and a reagent kit that was purchased from Roche. According to the manufacturer's instructions, detection was carried out.

Estimation of renal profile

Urea, creatinine, and uric acid were measured using an electrochemiluminescence immunoassay method on a fully automatic analyzer, the Roche Cobas 8000, with a Roche reagent kit. The detection was done according to the manufacturer's instructions.

Statistical analysis

Mean and standard error of the mean (SEM) were used to convey descriptive data. Body weight, glucose, and renal profile results were considered statistically significant if the P value was less than 0.05. One-way ANOVA was used to analyze the data for the body weight, glucose, and renal function tests, followed by a Tukey's *post hoc* test.

Results

3.1 Effect of CVS on the body weight (g) of rats

Table 1: Effect of CVS on the body weight (g)of rats

Group	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control	229.00 ± 1.90	230.50 ± 1.95	232.50 ± 1.61	233.50 ± 1.67	234.33 ± 1.74	235.00 ± 1.44	236.67 ± 1.65
CMS	226.67 ± 2.03	223.83 ± 2.01	223.00 ± 3.17*	221.17 ± 2.91**	217.83 ± 2.41***	217.33 ± 1.80***	215.33 ± 1.73***
CVS	224.50 ± 1.59	225.17 ± 1.92	225.33 ± 2.04	227.17 ± 1.78	229.00 ± $1.81^{\#}$	231.00 ± $1.81^{\#\#}$	232.33 ± $1.67^{\#\#\#}$
CMS + CVS	224.67 ± 2.06	222.17 ± 2.29	222.50 ± 2.16*	219.67 ± 2.70**	216.17 ± 3.03***$^{@@}$	215.67 ± 3.45***$^{@@@}$	214.33 ± 3.96***$^{@@@}$

Data are expressed as mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 compared with that of control. $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 compared with that of CMS group. $^{@@}$P<0.01 and $^{@@@}$P<0.001 compared with that of CVS group (One-way ANOVA followed by Tukey *post-hoc* test).

On day 14, 21, 28, 35 and 42, when compared with control group, CMS and CMS + CVS group showed significant decrease in body weight. When compared with CMS-induced group, on day 28, 35 and 42, increase in body weight was seen in CVS group. CMS +CVS group showed significant decrease in body weight on day 28, 35 and 42 when compared to CVS group as shown in table 1.

3.2 Effect of CVS on glucoseof rats

Table 2: Effect of CVS on glucoseof rats

Group	Glucose (mmol/L)
Control	5.02 ± 0.13
CMS	5.50 ± 0.28
CVS	5.13 ± 0.25
CMS + CVS	4.98 ± 0.26

Data are expressed as mean ± SEM. *P<0.05 and ***P<0.001 compared with that of control. $^{\#\#\#}$P<0.01 compared with that of CMS group (One-way ANOVA followed by Tukey *post-hoc* test).

There was no significant difference seen in glucose levels among all the four groups as shown in table 2.

Effect of CVS on renal profile of rats

Table 3: Effect of CVS on renal profileof rats

Group	Urea (mmol/L)	Creatinine (umol/L)	Uric Acid (mmol/L)
Control	5.32 ± 0.07	43.33 ± 1.28	0.09 ± 0.00
CMS	10.83 ± 0.67***	112.83 ± 2.01***	0.22 ± 0.03*
CVS	6.40 ± 0.50$^{\#\#\#}$	43.20 ± 1.67$^{\#\#\#}$	0.12 ± 0.02
CMS + CVS	5.18 ± 0.31$^{\#\#\#}$	46.25 ± 2.93$^{\#\#\#}$	0.20 ± 0.04*

Data are expressed as mean ± SEM. *P<0.05 and ***P<0.001 compared with that of control. $^{\#\#\#}$P<0.01 compared with that of CMS group (One-way ANOVA followed by Tukey *post-hoc* test).

Urea, creatinine, and uric acid levels significantly increased in CMS-induced group and uric acid level increased in CMS + CVS group when compared with that of control. When compared with CMS group, urea and creatinine levels decreased in CVS and CMS + CVS group as shown in table 3.

Discussion

The purpose of this study was to examine the role of CVS on body weight, blood glucose, and renal profile, in CMS-induced rats. This study showed that CVS can be employed to alter CMS-induced alterations in the body weight, glucose, and renal profile. CVS prevented CMS-induced changes in the aforementioned parameters.

Stress is defined as the response of the body to pressure from a specific circumstance or incident. Stress should be regarded as a physiological and behavioral reaction to the cognitive notion of unpredictability and/or uncontrollability. Stress varies from the most mildly challenging stimulation to profoundly unpleasant conditions (Koolhaas *et al.*, 2011). Stress consists of both a stressor and a stress response. A stressor is the stimulation that endangers homeostasis, and the stress response is the body's response to that stimulation in an effort to restore balance (Chrousos., 2009). CMS was utilized in this study as it mimics the effects of stress in humans. CMS has fairly consistent impacts on neurobiological indicators that reflect synchronous changes in clinical groups with severe depressive illness or suicide victims. The fact that this reduction in hedonic impact was reversible by prolonged therapy with standard antidepressant drugs, which paralleled the time course required for the clinical effectiveness of those medications, underlined the validity of this concept (Willner., 2005, Willner *et al.*, 1987). In the present study, CVS was used to modulate the body weight, glucose and renal profile in CMS-induced rats.

The body weight of the rats revealed that when compared with the control group, the CMS and CMS + CVS group showed a significant decrease in body weight. Previous studies reported that in times of stress, little over a quarter of people eat less than normal, whereas 35 percent to 60 percent say they eat more. The kind and/or degree of the stressor, prior eating experiences, or reward reactions to stress/food have all been put up as potential causes for this differential response. Studies on humans have shown that low-mild stressors may result in increased food consumption(Rutters *et al.*,

2009) whereas high-intensity stressors are associated with decreased food intake (Popper *et al*., 1989).Two independent aspects of eating are involved: one mediates the consumption of food to maintain energy balance (homeostatic eating), and the other involves the pleasant aspect of eating. Additionally, stress may alter both homeostatic and hedonic eating (Chami *et al*., 2019). When compared to the CMS group, the CVS group showed a considerable improvement in body weight, demonstrating that CVS has a positive influence on preserving body weight to normalcy.

The blood glucose levels in the rats of all the groups did not show any significant difference when compared with both the control and CMS group demonstrating that CVS is a preventative approach for preserving normal blood glucose levels. In non-diabetic rats, the blood glucose levels are functionally managed by the glucoregulatory system and the negative feedback control systems very tightly. In diabetic rats, stress can deteriorate glycemic control and spike blood glucose levels (Wing *et al*., 1985) As this study did not employ diabetic rats such changes were not observed.

The assessment of urea and creatinine is used to reflect the glomerular filtration rate (GFR) whereas uric acid is used to diagnose gout.Urea, creatinine, and uric acid levels considerably increased in the CMS-induced group and uric acid levels increased in CMS + CVS group when compared with that of the control indicating the existence of some renal impairment (Koepke., 1989). When compared with CMS group, urea and creatinine levels dropped in the CVS and CMS + CVSgroup denoting that CVS is a modest strategy that can be employed to reverse the deleterious effects of CMS on kidney function and restore normal kidney function.

Conclusion

Adverse stressors can elicit varying degrees of stress, which can increase a person's biological susceptibility to a wide variety of psychological conditions. By reinstating the body's balance to normal, the CVS model has revealed to be a very valuable tool for reducing the consequences of CMS on body weight, blood glucose, and kidney function. A reliable and effortless non-invasive therapy that can be advocated in daily life to attain a stress-free existence is caloric vestibular stimulation.

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