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Anatomy & Physiology: Current Research

Research Article

A Study of the Effect of Auditory Stimuli on GLP1, Insulin, Liptin and Body Weight of Crowded Adult Albino Rats

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ABSTRACT

Background: Music therapy is an established health profession in which music is used within a therapeutic relationship to address physical, emotional, cognitive and social needs of individuals. Stress is the reaction of the organism to stimulus which changes psycho-physiological equilibrium with harmful consequences. This disturbance leads to changes in the concentration of large number of different hormones that have a crucial role on homeostasis.

Objectives: The current study was designed to clarify the possible effect of enhancing the quality of environment to alleviate the stressful environmental condition.

Material and Methods: Forty-five adult male albino rats were chosen for this study. They were divided into five groups as follows:

Group 1: Served as normal control rats.

Group 2: Rats exposed to crowding.

Group 3: Rats exposed to crowding and listening to instrumental music.

Group 4: Rats exposed to crowding and listening to soft song.

Group 5: Rats exposed to crowding and listening to rallying song.

After 90 days all rat groups were anesthetized, and blood sample collected for measurement of Glucagon Like Peptide 1(GLP1) level, Insulin level, Leptin level and specimens from pancreas were fixed for histopathological examination for these groups and all pancreas sections were examined using light microscope.

Results: Music has no significant ($P<0.05$) effect on water and food consumption but it has significant ($P<0.01$) effect on body weight in music listening groups in comparison with control crowded group. Music listening can significantly ($P<0.01$) increase level of GLP1 and leptin but significantly ($P<0.01$) decrease level of insulin in comparison with control uncrowded group.

Conclusion: Music may alleviate the stressful situation and improve quality of life.

Keywords: Music; Stress; Crowding; GLP1; Insulin; Liptin; Body weight

INTRODUCTION

Music is the art of combining vocal or instrumental sounds or both to produce beauty of form, harmony, and expression of emotion [1]. Rhythm is the basic character of music. In addition, almost all physiological functions go in arrhythmic pattern. Rhythm can be felt e.g. in the heart beats, walking and breathing etc. the essence of rhythm is a recurring pattern of tension and release. This has to be regulated in music by harmony which means that the rhythmicity of different sounds has to go together and follow each other changing in the tone color create variety and contrast the sounds more [2]. Response to music is multifactorial according to objective characteristics of music e.g. Intensity of sound, type of sound, tone.....etc. and subjective factors on individual e.g. preferences, mood or emotions [3,4]. Music has consistent physiological responses e.g. cardiovascular, respiratory, hormonal and nervous responses with different styles [5]. Fast music may increase arterial blood pressure, heart rate and breathing rate, and reduced baro-reflex sensitivity. Slow music, on the other hand, may

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Received: 20-Sep-2022, Manuscript No. APCR-22-19296; **Editor assigned:** 22-Sep-2022, PreQC No. APCR-22-19296 (PQ); **Reviewed:** 10-Oct-2022, QC No. APCR-22-19296; **Revised:** 21-Oct-2022, Manuscript No. APCR-22-19296 (R); **Published:** 01-Nov-2022, DOI: 10.35248/2161-0940.22.12.391

Citation: El-Rhman AAA, Al-Etreby MZ, Khalil AA, Fawzy M, Mansour AE (2022) A Study of the Effect of Auditory Stimuli on GLP1, Insulin, Liptin and Body Weight of Crowded Adult Albino Rats. Anat Physiol.12:391.

cause a significant fall in heart rate and breathing frequency compared with the baseline [6]. The physiological effects exerted on nervous system may be relaxing or exciting [7,8]. Stress is a state in which the brain interprets the quantity of stimulation as excessive or its quality as threatening [9]. Stress may be physical, chemical or emotional to which an individual fail to adapt [10]. Stress consequences occur when living organism fails to cope with emotional or physical threats [11]. Stress is a multifactorial phenomenon. Psychological, physiological and environmental factors, stress affect homeostasis through the Hypothalamic-Pituitary-Adrenal (HPA) and the hypothalmao-sympathetic axes [12]. Appetite is the desire to eat and it's essential for food intake and for maintaining weight and health in general [13]. Appetite includes conscious and unconscious urges to eat and affected by preceding meals, environment and learning than by BW [14,15]. Nutrition is the food intake in relation to body needs and it is fundamental for all life processes including growth and health. It is important even in patients with chronic diseases who are affected by abnormal inflammatory and metabolic processes that can lead to malnutrition [16,17]. Chronic stress may increase or decrease appetite, which is a common clinical problem in patients with chronic diseases. Various definitions can be used to explain decreased appetite, such as anorexia that in turn is commonly referred to loss of appetite and reduction or lack of the sensation of hunger or lack of desire to consume food and/or decreased food intake [18-20] also appetite is controlled by Intestinal peptides and hormones like ghrelin, Glucagon Like Peptide 1(GLP1), Peptide YY (PYY), leptin, cholecystokinin and glucose dependent insulinotropic polypeptide [21,22].

MATERIALS AND METHODS

Experimental animals

Forty-five adult male albino rats were chosen as an animal model for this study with initial body weights ranging from 140-160 g. Rats were purchased from animal house of Al-Azher University, Cairo, Egypt. They were maintained on standard diet of known composition, and water supply in clean containers and the animals were housed in standard polypropylene cages with stainless steel base, good aerated conditions and maintained under room temperature with normal light-dark cycle [23]. They were fed on the standard food prepared from commercial rat food formula (El-Nasr-Pharmaceutical Co.) in addition to bread and green vegetables with free water supply. They were kept for two weeks to adapt to the laboratory conditions before the start of the experiment in faculty of medicine, Al-Azhar University (Cairo branch), Physiology Department.

Kits

Glucagon Like Peptide 1 (GLP1) level was determined by using specific ELISA kit, (EZGLP1T 36K; Merck Millipore, Darmstadt, Germany) according to the manufacturer instructions. Insulin levels were determined by using specific ELISA kits (AKRIN-010T; Shibayagi Company Limited, Gunma, Japan). Leptin levels were determined by using sandwich ELISA kit (Germany), according to the manufacturer instructions.

Study design

The experimental animals were divided into five groups; each group is comprised of nine rats as follows:

Group 1: Served as normal control rats 3 rat/cage (25 ×30 × 25 cm).

Group 2: Rats exposed to crowding 9 rat/cage (25 × 30 × 25 cm) only for 90 consecutive days.

Group 3: Rats exposed to crowding and was listening to instrumental music (Samaei Agam) for 90 consecutive days, 1 h/day.

Group 4: Rats exposed to crowding and was listen to soft song (Zoroni Kol Sana Mara, Fayrouz-Maqam Agam) for 90 consecutive days, 1 h/day.

Group 5: Rats exposed to crowding and was listen to rallying song (Anthem Allah Akbar-Maqam Agam) for 90 consecutive days, 1 h/day.

Application of crowding S

Crowding was induced by multiplication of normal density × 3 i.e. 9 rat/cage. A group of 9 rats put in a cage (25 ×30 × 25 cm) [24].

Induction of music

Music was induced by exposure of the tested animals to a prerecorded instrumental music, soft song and rallying song of sound intensity 60 db delivered *via* speaker placed one meter from the cages one hour daily. Every group was put in a separate room away from other rats for 90 consecutive days [25].

Statistical methods

The mean, the standard deviation, and percentage were calculated. The obtained data were subjected to analysis of variance one-way and three-way according to the procedures outlined by Snedecor, et al. [26]. The mean value of treatments was compared according to Least Significant Difference (LSD). The data was analyzed using CoStat software for windows (version 6.3).

RESULTS

Water and food intake

Table 1 shows significant ($P<0.01$) difference of the amount of food and water consumption between uncrowded and crowded rat groups. Music shows no significant ($P<0.05$) difference of the amount of food and water consumption between all groups

Body weight level among different groups as compared with control group in the 1st and 13th week.

Table 2 shows that at the 1st weak, body weight of control uncrowded group was 152.17 ± 7.05, body weight of control crowded group was 151.67 ± 4.87, body weight of crowded group listening to instrumental music was 157.89 ± 11.83, body weight of crowded group listening to soft song was 151.22 ± 5.67 and body weight of crowded group listening to rallying song was 151 ± 12.82 with insignificant ($P<0.05$) difference between all rat groups. After 13th weak, body weight of control uncrowded group was 231.17 ± 18.83, body weight of control crowded group was 154.7 ± 9.46, body weight of crowded group listening to instrumental music was 186.5 ± 13.57, body weight of crowded group listening to soft song was 178.7 ± 13.82 and body weight of crowded group listening to rallying song was 164.8 ± 18.29 with significant ($P<0.01$) difference between all rat groups.

Hormones

Table 3 shown that Body Mass Index (BMI), S GLP1 mol/L, S insulin IU/ML and S leptin ng/ml of rats were significantly affected by crowding and music whereas (P-value<0.01).

Table 1: Water and food amount.

Animal groups	Music effects	Food amount		Water	
		Week			
		1st	13th	1st	13th
Uncrowded	Control	34 gm/day	34 gm/day	56 cm³	56 cm³
Crowded	Control	15 gm/day	15 gm/day	20 cm³	20 cm³
	Instrumental	15 gm/day	15 gm/day	20 cm³	20 cm³
	Soft song	15 gm/day	15 gm/day	20 cm³	20 cm³
	Rallying song	15 gm/day	15 gm/day	20 cm³	20 cm³

Note: LSD=00.00

Table 2: Body weight.

Animal groups	Music effects	Week	
		1st	13th
Uncrowded	Control	152.17 ± 7.05	231.17 ± 18.83
Crowded	Control	151.67 ± 4.87	154.7 ± 9.46
	Instrumental music	157.89 ± 11.83	186.5 ± 13.57
	Soft song	151.22 ± 5.67	178.7 ± 13.82
	Rallying song	151 ± 12.82	164.8 ± 18.29

Note: LSD=14.01

Table 3: Body Mass Index (BMI), S GLP mol/L, S insulin IU/ML and S leptin ng/ml of rats among different groups as compared with control group.

Animal groups	BMI	S GLP1 mol/L	S Insulin IU/ML	S Leptin ng/ml
P-value	0.00**	0.00**	0.00**	0.00**
Control Uncrowded	0.64 ± 0.02	11.6 ± 2.2	22.9 ± 3.3	0.58 ± 0.05
Control crowded	0.44 ± 0.03	25.8 ± 3.8	10.4 ± 2	0.66 ± 0.02
Crowded listening to instrumental music	0.51 ± 0.03	17.7 ± 2.2	18.5 ± 3.9	0.8 ± 0.03
Crowded listening to soft song	0.51 ± 0.06	18.9 ± 2.8	16.1 ± 4.3	0.75 ± 0.03
Crowded listening to rallying song	0.47 ± 0.06	19.1 ± 3.9	15.2 ± 3.2	0.67 ± 0.02

Note: ** and NS indicate significant at 0.01<0.01 and insignificant at 0.05, respectively. Means followed by a common letter are not significantly different at the 1% level by LSD.
Mean ± Standard deviations

Histopathology

Examining tissues under a microscope (Figures 1-9).

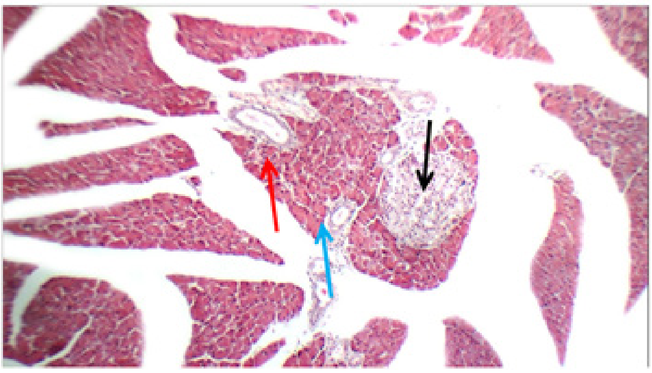

Figure 1: (Control uncrowded): Pancreas showing average-sized pale-staining islets of Langerhans (black arrow), average exocrine areas (blue arrow), and average ducts (red arrow) (H&E X200).

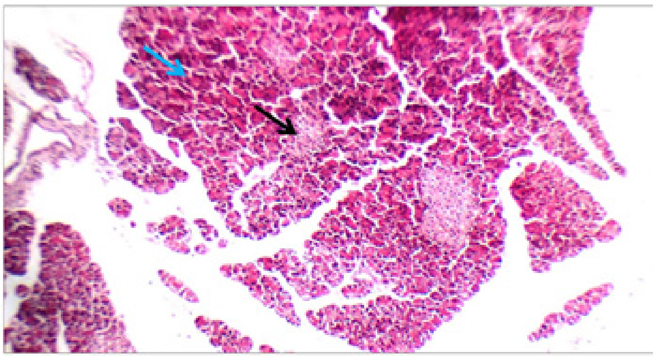

Figure 2: (Control crowded): Pancreas showing scattered small-sized pale-staining islets of Langerhans (black arrow), and average exocrine areas (blue arrow) (H&E X200).

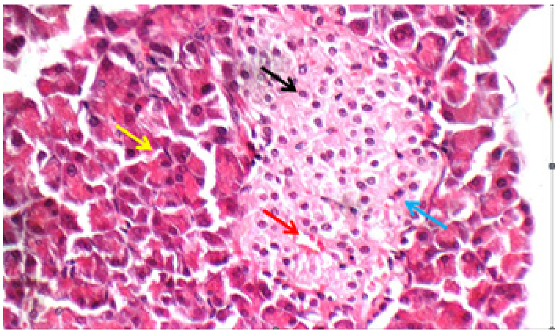

Figure 3: (Control crowded): High power view showing average beta cells with pale blue cytoplasm (black arrow), average alpha cells with pink cytoplasm (blue arrow), average intervening blood capillaries (red arrow), and average exocrine area (yellow arrow) (H&E X400).

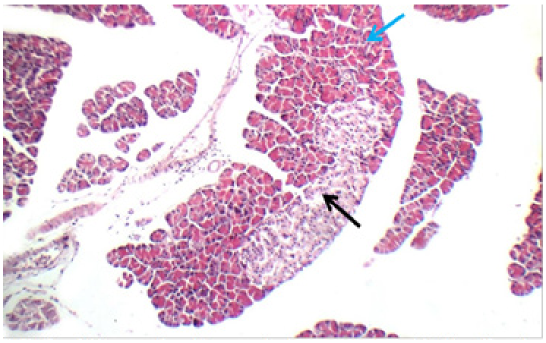

Figure 4: (Crowded instrumental music): Pancreas showing average-sizedpale-staining islets of Langerhans(black arrow), and average exocrine areas (blue arrow) (H&E X200)

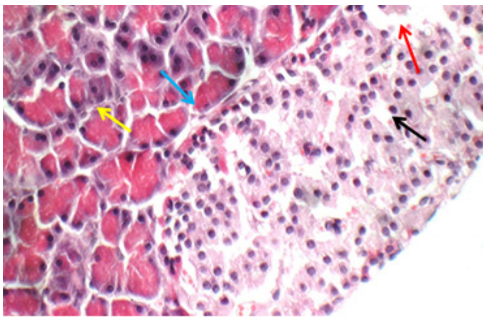

Figure 5: (Crowded instrumental music): High power view showing average beta cells (black arrow), average alpha cells (blue arrow), mildly dilated intervening blood capillaries (red arrow), and average exocrine area (yellow arrow) (H&E X400).

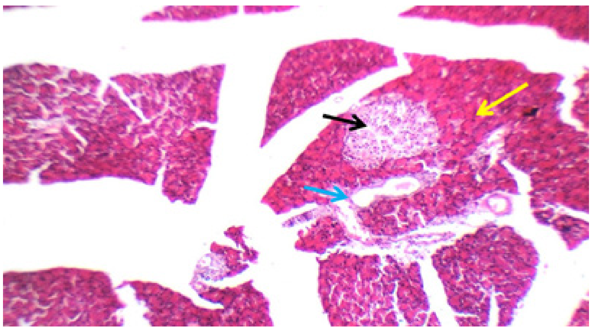

Figure 6: (Crowded soft song): Pancreas showing average-sizedpale-staining islets of Langerhans (blackarrow), average duct (blue arrow) and average exocrine area (red arrow) (H&E X200).

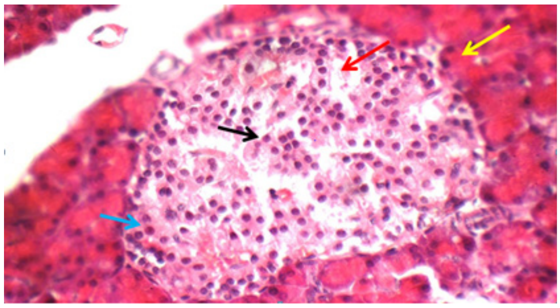

Figure 7: (Crowded soft song): High power view showing average beta cells (black arrow), average alpha cells (blue arrow), mildly dilated intervening blood capillaries (red arrow), and average exocrine area (yellow arrow) (H&E X400).

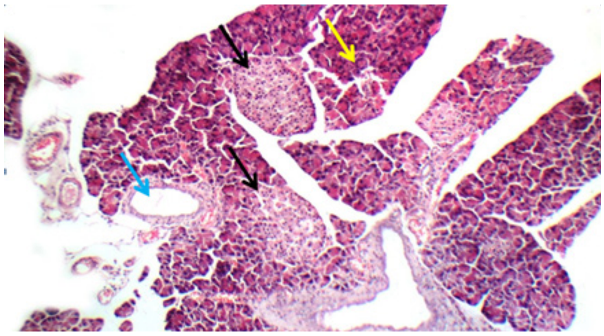

Figure 8: (Crowded rallying song): Pancreas showing average-sizedpale-staining islets of Langerhans (black arrow), average ducts (blue arrow), and average exocrine areas (yellow arrow) (H&E X200).

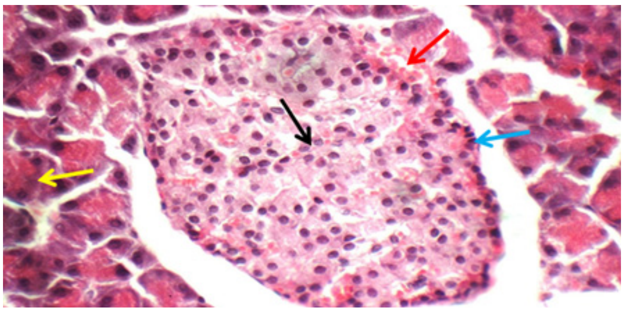

Figure 9: (Crowded rallying song): High power view showing average beta cells (black arrow), average alpha cells (blue arrow), mildly congested intervening blood capillaries (red arrow), and average exocrine area (yellow arrow) (H&E X400).

DISCUSSION

The current study was designed to clarify the possible effect of enhancing the quality of environment to alleviate the stressful environmental condition. Stress in general particularly crowding is considered as problems of many modern communities and work environments [27]. Crowding exposure activates the autonomic and hormonal systems leading to temporary changes such as increased blood pressure, heart rate and vasoconstriction. After prolonged exposure susceptible individuals may develop permanent effects such as increase or decrease body weight, hypertension and ischemic heart disease due to cumulative effect of stress, Tomoyuki, Eid, et al. Liu, et al. and Karapetsa, et al. mentioned that music enables a person to gain valued emotional experiences and may affect eating behavior [28-31]. Also Popa, et al. and Situmorang, et al. mentioned that music can be used as an adjuvant therapy in the medical field to improve health and behavior due to its good emotional effect [32,33], Goldschmidt, et al. and Paoli, et al. mentioned that food intake and eating behavior are influenced by internal physiological factors and external environmental factors [34,35]. Regarding to food consumption of developing rats in our study the food consumption increased in rats with increasing age and remained constant thereafter in all groups. The food consumption is increased significantly (P<0.01) in uncrowded group in comparison with the crowded groups. These finding are in agreement with the finding of Zouridis, et al. who reported that food consumption significantly increases during growth then remains constant [36]. The results of the present study demonstrated that neither type of musical template appeared to have an effect on food intake in all groups consistent with the results of Stroebele, et al. and Hauck, et al. who reported that neither volume nor speed of music appeared to have an effect on food intake on rats [37,38]. Previous studies have reported significant effects of volume and speed of music on food intake were in human in a restaurant environment [39,40]. The results of the present study demonstrated that body weight of crowded control rat group decreased significantly (P<0.01) in comparison with the control uncrowded rats and body weight of crowded rat groups listening to instrumental music, soft song, and rallying song respectively. The most decrease of body weight was in crowded control rat group followed by crowded rat groups listening to rallying song, soft song and instrumental music respectively in comparison with control rat group. These results are in agreement with the findings of Caine, Standley and Russo, et al. [41-43] who found that music increased significantly body weight of rats contradicting this result with Teckenberg, et al. Malloch, et al. and Standley, et al. who found that no significance in weight changes effected by music therapy [44-46]. Also Finger, et al., Slattery, et al. and Geiker, et al. reported that body weight was reduced during the presence of stressor [47-49]. Also Slattery, et al., Melhorn, et al. and Cassidy, et al. reported that increased body weight gain upon termination of the stressors [48,50,51]. The present study's observations showed that there was a significant (P<0.01) reduction in food and water consumption behavior in all experimental animals when exposed to crowding stress in comparison with uncrowded rat group and it lead to a reduction in body weight in the same duration. So, the reduction in weight loss could be associated with the reduced intake of food and water during that specific duration. Our results were correlated to the reported finding of Hariram, et al., Sundar, et al. and Jacques, et al. [52-54] who reported that reduced food and water consumption could be a reason for sudden weight loss during stress. The loss of body weight in animals and decreased food consumption in the present study may be attributed to decrease insulin level which is an anabolic hormone that leads to decrease body weight. Our results were correlated to the reported finding of Valencia, et al. and Gądek-Michalska, et al. who reported that exposure of rats to crowding stress showed activation of HPA with subsequent release of adrenocorticotrophic hormone, cortisol and catecholamines leading to increased glucose level and decreased insulin level [55,56]. Also our results showed an increase in the level of leptin hormone in all crowded rat groups which in turn lead to decrease appetite and food intake leading to decreased body weight. Our results were correlated to the reported finding of Wang, et al. and Sze, et al. who reported that crowding stress stimulating the synthesis and secretion of adipocyte derived leptin which regulates food intake and energy expenditure [57,58]. Also there is an increase in the level of GLP1 hormone level in all crowded rat groups which decrease food intake leading to loss of body weight. Our results were correlated to the reported finding of Reiner, et al. and Zizzari, et al. [59,60] who reported that GLP-1 controls food intake and modulating the energetic balance by acting on GLP-1R in a multitude of energy balance-relevant nuclei in the hypothalamus but also in other mesolimbic brain areas involved in reward system. Also, Williams, et al., Décarie-Spain, et al. and Samms, et al. mentioned that GLP1 reduces meal size in rats and humans and increases inter meal intervals accounting for its suppressive effect on food intake [61-63]. Music template appeared to be an important potential moderator in our study where the effect of listening to rallying song differs from soft song and instrumental music respectively. In general, the food consumption rate seems to be influenced by the template of music as reported by Spence, et al. and Huang, et al [64,65] who reported that drinking and eating faster when fast music was played than in a slow music. Wilczek, et al. and Wang, et al. reported that diners who ate while they were listening to classical music enjoyed more their dinner in comparison to diners that ate without back-ground music [66,67]. Kantono, et al. and Mathiesen, et al. mentioned that food intake may be affected by volume dynamics of music and high volume music increases food and drink consumption [68,69]. Moreover, Wansink, et al. and Biswas, et al. mentioned that music genre is another factor that affects eating behavior [40,70]. This can explain the significant difference in results between different rat's groups conducted in our study due to different music template. Pancreas morphology was in accordance with Pan Anderson, et al. and Kasajima, et al. described that pancreas is composed of average-sized islets of Langerhans composed of predominating beta cells in center and alpha cells in the periphery separated by average thin-walled blood capillaries, average exocrine areas and average ducts as in Figure 1 [71,72]. These results are in agreement with those of Pan Anderson, et al. and Raghav, et al. who reported that stress induced free radicals readily damage the beta cells of the islets and pancreatic islets cells showed lymphocytic infiltration as in Figures 2 and 3 [71,73]. These findings are important because pancreatic β-cell dysfunction, characterized by decreased insulin secretory capacity due to insufficient β-cell mass and/or functional defects of the β-cells [74-76]. These morphological changes of pancreatic tissue in different rat groups can explain different insulin level among these groups.

CONCLUSION

Biochemical and histological findings of the present study indicate that crowding stress is dangerous psychosocial problem that affect the health and quality of the life. In addition, music may alleviate and prevent the complication of crowding stress. In conclusion it seems that music has a great benefit on human health. Moreover, our study revealed that different music template can exert different cumulative effects on body weight and may have no effect on food and water consumption. Response of albino rats to auditory stimulus in general and music may be biological in nature.

RECOMMENDATIONS

Further studies are required for further evaluation and establishment of the beneficial effects of different music template in alleviation and prevention of hazards of crowding stress and stress in general and enhancement of the quality of environment.

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