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Original Article

Alcohol Drinking Behavior in Rats Exposed to Crowding Stress

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ABSTRACT **One of the environmental factors influencing the animals to drink alcohol is stress or arousal and housing. There is considerable evidence showing that housing and social factors influence alcohol intake in rats. The aim of this study was to find out the effects of crowding stress on voluntary alcohol intake in rats and also to observe whether there is any ender difference in free choice alcohol intake. Wistar strain albino rats of either sex were exposed to one week crowding stress and the effect of crowding on voluntary alcohol consumption, alcohol preference (%) and total alcohol intake (g/kg body weight) were studied. There was an increase in the alcohol preference and alcohol intake after one day stress compared to seven days stress in male rats. Male rats showed an adaptation to alcohol intake over one week period. A significantly higher alcohol intake was observed in female rats than the male rats after 7 days stress and there was no adaptation. Tus, there is an increase in voluntary alcohol drinking behavior after chronic crowding stress in female rats compared to male rats.**

Key words: **Alcohol, crowding, stress, alcohol preference, rats**

Exposure to stressful situations is among the most common human experiences. Regardless of their degree of severity, stressors may promote physiological and behavioral disturbances ranging from psychiatric disorders to immune dysfunction[1,2]. Stress is one of the deleterious factors for health. Many diseases or disorders such as hypertension, gastric ulcer etc are related to stress[3]. There is a complex relationship between alcohol consumption and stress. Not only stressful situations induce drinking, but alcohol onsumption also has long been considered a way of relieving stress[4]. Alcohol consumption can reduce the magnitude of an organism's response to stress and this has been described as stress response dampening (SRD) effect[5]. Numerous studies have found that stress increases alcohol consumption in animals and that the individual animal may differ in the amount of alcohol that they consume in response to stress[6,7]. Data concerning gender dependent effects of stressors and ethanol intake are relatively limited, although researchers have found that the hypothylamus – pituitary – adrenal response to tressors is greater in females than in males. This may contributes to the gender differences often seen with respect to some behavioral disturbances (e.g. mood disorders), but contribution of these factors to alcohol or other drug (AOD) consumption is not yet clear.

There are several studies reporting the effect of

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crowding stress on exploratory behavior in rodents, whereas, the information is lacking regarding the effect of crowding stress on feedign behavior and voluntary ethanol consumption[8,9]. Thus the purpose of the present work was to elucidate the effect of overcorwding environment on food intake, body weight and voluntary ethanol consumption in albino rats of either sex with research hypothesis that there is a gender difference in stress induced voluntary ethanol consumption in albino rats.

MATERIALS AND METHODS

Inbred adult albino rats of Wistar strain (12 males and 12 females) weighing between 250-300g at the start of the experiment were used for this study. Before the start of the experiment, rats did not have any experience in intake of any other liquid beside tap water. Rats were maintained on a 12-12 hr light dark cycle under standard laboratory conditions. Food pallets were available ad libitum during all the conditions of the study. The animals were housed in groups of two per care for two weeks to adapt to the novel laboratory conditions. The animals were divided into following groups;

Control Group I: Consisted of six male rats kept in the standard laboratory conditions without any stress exposure.

Control Group II: Consisted of six female rats kep under standard laboratory conditions without any stress.

Control animals were kept in polypropylene cages with two animals per cage.

Crowding Sterss Group I: Six male rats were kept in a single polypropylene cage in a way that minimum mobility was possible for the animals inside their cage. The rats were kept in this overcrowded condition for seven days.

Crowding Stress Group II: Six female rats were kept in the overcrowded condition as mentioned above. Crowding stress for female rats also continued up to seven days.

The animals in the control and stressed group had continuous access to two bottle fluid sources. One bottle containing tap water and the other containing 5 percent (w/v) alcohol solution made from 95 percent ethanol mixed with tap water. Positions of the bottles were randomly altered daily to prevent place preference. All the animals had free access to food throughout the duration of the experiment.

Body weight, fluid consumption (both alcohol and water) and food intake were monitored daily between 10AM to 11AM. Whole body weight was expressed as body weight changes in grams from the initial body weight before the start of the experiment. Fluid intake was recorded by measuring remaining fluid content in the bottle. 24 hours food consumption was measured by weighing what was left behind in the cage, of the food provided on the previous day. Daily alcohol intake (total alcohol intake) was calculated in grams per kilograms body weight. Alcohol preference was calculated taking percentage of total fluid intake drunk as alcohol (alcohol take/total fluid intakeX100). Experimental protocols and facilities were reviewed and determined to meet the requirements of the institutional animal care and use committee.

Statistical Analysis – data collected were computed for mean values ± standard error of the mean. Comparison between control and stressed groups and between males and females were based on Mann Whitney "U" test. Analysis between one day and seven days were done by Friedman's test. *P* value of less than 0.05 was considered statistically significant.

RESULTS

Friedman's test analysis revealed a significant increase in the alcohol preference after one day crowding in male rats ($P < 0.001$). In females, a significant increase in the preference was seen after 7 days stress compared to one day stress ($P < 0.01$). A similar significant difference was observed in male rats between one day and 7 days stressed groups ($P < 0.001$). After oen day stress also, females had significantly lower preference than male rats ($P < 0.01$) [Table 1].

Stressed male rats did not show any significant difference in the alcohol intake compared to control rats. Whereas, a significant increase in totla alcohol intake was observed after one day stress compared to 7 days stress in male rats ($P < 0.01$). A gradual increase in the total alcohol intake was seen in female rats from day one to 7 days after crowding stress. There was a significant difference in the alcohol intake after 7 days stress exposure compared to one day stress ($P < 0.001$) [Table 1]. After one day stress, male rats showed a significantly higher alcohol intake than female rats for the same duration

($P < 0.001$). But after 7 days, totla alcohol intake significantly increased in female rats ($P < 0.001$). Female stressed group had significantly higher alcohol intake than male rats when compared to one day and 7 days duration (Table 1).

Food intake decreased significantly after one day satress ($P < 0.001$) compared to the control group of male and female rats. The food intake improved gradually after first day of stress and after 7th day the food intake was significantly higher than one day stress in both male and female rats ($P < 0.001$) (Table 2). When food intake was compared between male and female rats, a significant difference in the food intake was seen after one day exposure to the stressor. Female rats in the overcrowded ambience consumed significantly less food ($P < 0.01$) than the male rats after one-day crowding stress (Table 2).

Body weight decreased significantly after one day crowing stress in both male and female rats ($P < 0.001$). As the stress period was prolonged, there was a gradual recovery in the body weight, and after 7 days of stress exposure, the body weight increased significantly,m greater than one-day stress exposure ($P<0.05$) (Table 2). When body weight changes between males and females were compared, there was no significant changes were observed after stress exposure.

DISCUSSION

Stress response is a complex process; the association between drinking and stress is still more complicated. Both drinking behavior and an individual's response to stress are determined by multiple genetic and environmental factors. Studying the link between alcohol consumption and stress may further our understanding of drinking behavior[6,10]. In the present study, one week crowding stress increased alcohol consumption in the female rats where as, the total alcohol intake (g/kg body weight) was significantly less in male rats. Alcohol preference (%) was significantly more in male rats after one-day stress and the preference decreased gradually afterwards. Hence more significant increased in the total alcohol intake and preference was there in male rats

Table 1 Alcohol preference and total alcohol intake after crowding stress

Parameters	Control Males (n = 6)		Control Females (n = 6)		Stressed males (n = 6)		Stressed Females (n = 6)	
	1 day	7 days	1 day	7 days	1 day	7 days	1 day	7 days
Alcohol preference (%)	57.83± 3.30	56.00± 2.91	63.17± 3.67	44.17± 2.09	76.17± 1.33!!**††	55.67± 1.25!	43.87± 0.64	47.75± 0.70††
Total alcohol intake (g/kg b.wt)	2.01± 0.46	1.60± 0.47††	2.76± 0.27!	1.98± 0.19	2.07± 0.13††!!	1.17± 0.07	1.20± 0.13	3.82± 0.29$^{**\dagger\dagger!!!}$

Values are Mean ± SEM, n = Number of animals; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\#}P < 0.001$ - Control with stressed groups; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ – One day with 7 days; $!P < 0.05$, $!!P < 0.01$, $!!!P < 0.001$ – Male with female

Table 2 Body weight changes and food intake after crowding stress

Parameters	Control Males (n = 6)		Control Females (n = 6)		Stressed males (n = 6)		Stressed Females (n = 6)	
	1 day	7 days	1 day	7 days	1 day	7 days	1 day	7 days
Body weight	–1.33± 0.42†	2.67± 0.73	–1.67± 0.31	2.00± 0.42	-8.00± 0.15††	1.33± 0.84	-10.75± 1.53*	- 1.50± 0.75†
Food intake (g/100g b.wt)	6.56± 0.98	7.05± 0.49	7.51± 0.65	7.18± 0.96	2.52± 0.22$^{\#\dagger\dagger\dagger}$	7.55± 0.53	1.83± 0.23$^{\#\dagger\dagger\dagger}$	7.24± 0.71

Values are Mean ± SEM, n = Number of animals; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\#}P < 0.001$ – Control with stressed groups; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ – One day with 7 days; $!P< 0.05$, $!!P < 0.01$, $!!!P < 0.001$ – male with female

only in the inditial period of stress exposure. Female rats showed increase in alcohol consumption even after 7 days of stress exposure. Several studies have found that the individual animals differed in the amount of alcohol they consumed in response to stress[7,11]. Animal studies reporting a positive correlation between stress and alcohol consumption suggest that drinking may take place in response to stress perceived as unavoidable.

In the present study, male rats exposed to 5 percent (w/v) ethanol did show an increased voluntary consumption in the initial stages of stress expsoure. But these rats showed adaptation to the alcohol when they were provided with choice between water and alcohol over a period of one week. Famale rats continued to consume more alcohol throughout the duration of stress and no such adaptation to alcohol was observed. In has been reported that men consume alcohol more frequently and in larger amounts than women[12,13]. In our study, the alcohol consumption in female stressed rats was more than in stressed male rats. The higher alcohol intake in female rats after exposure to stressor could be attributed to the difference in pharmacokinetics of alcohol. There are sex differences related to pharmacokinetics of alcohol that may influence differentially drinking patterns of alcohol. First, metabolic rate of alcohol seems to be higher in females than in males. Second, rates of first pass alcohol metabolism, related to gastric alcohol dehydrogenase appear to be lower in women than men. Third, it is well known that females have less body water than males, this is important because ethanol distributes from blood into the issues and fluids in proportion to their relativet contents of water, therefore, volume distribution of ethanol in the body is equivalent to the total body water[14-16] have postulated about the possibility of neurosteroid activity, playing a significant role in producing differential alcohol effects in males and females. In the case of social stress, stressors are not physical events but arise from the immediate social environment of the animal. The presence and the activities of co specifies often force animals kept in groups to cope with adverse conditions and can exert detrimental effects, resulting in reduced fitness. Stress induced decrease in body weight was more marked after one-day stress exposure, both in male and female rats. There was regain in body weight after 7 days exposure to the stressor. The recovery back to normal body weight after seven days could be due to the adaptation to the stressful stimulus. Food intake decreased significantly in the initial period of exposure to stressor and there was recovery in food intake after 7 days stress. The decrase in the body weight after stress exposure could be due to attributed to the hypophagia as a result of stress. Various physiological changes seen in response to stress are primarily due to the activation of hypothalamic pituitary adrenal system[17]. One week of crowding is known to stimulate pituitary adrenal system[18]. Researchers have found that the HPA response to stress is greater in female rats than in male rats. This affect appears to occur at almosat every level of HPA functioning and the responses to some extent are regulated by interaction among the hypothalamus, pituitary gland and gonadal organs[19]. hence, the increased corticotropin releasing hormone (CRH) in females could be the cause for more suppressed food intake and more reduction in body weight after exposure to acute crowding stress.

Thus, the present work confirms that crowding stress decreases the body weight gain and food intake in the initial period of exposure to stressor. There was an adaptation or habituation to the stressor after 7 days where the animals regained weight and oof intake. Male rats showed an increased preference for alcohol only in the initial period of stress exposure after one day stress but the total alcohol intake was significantly more in females throughout the duration of stress. We can not ignore the fact that this consideration could be intimately related to patterns of alcohol consumption which can change from time to time in male and females independent of the quantum of alcohol consumed. The present work shows that the female rats show an increased voluntary drinking behavior on exposure to stress than in male rats. These results encourage further examination of the physiological mechanisms in female rats during stress that influence alcohol drinking behavior.

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