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# EFFECT OF ACUTE AND CHRONIC CONDITIONS OF OVER – CROWDING ON FREE CHOICE ETHANOL INTAKE IN RATS

H. S. NAGARAJA* AND P. S. JEGANATHAN

*Department of Physiology,*
*Kasturba Medical College,*
*Center for Basic Sciences,*
*Bejai, Mangalore – 575 004*

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**Abstract :** Male albino rats of Wistar strain were exposed to overcrowding stress in two different groups for a period of seven days. One group of rats was kept under stress for six hours per day (acute stressed group) and the other group rats was kept under stress continuously (chronic stressed group). The effect of these acute and chronic stresses on voluntary alcohol (2% w/v) intake was monitored during the 7 days of stress exposure, and ethanol preference and total ethanol intake in terms of g/kg body weight were also studied. A significant increase in ethanol preference and ethanol intake was observed in one–day and 7 days chronic stressed group. No significant increase in ethanol intake was observed in acute stress. Thus a short lasting stressor may not increase ethanol –drinking behavior, whereas when animals were exposed to more intense stressor continuously for 7 days, an increase in voluntary drinking behavior may be seen.

**Key words:** ethanol crowding acute stress chronic stress

## INTRODUCTION

Stress is thought to be a contributing factor to human illness including cardiovascular disease, gastrointestinal dysfunction and mental illness. Stressors are classified into acute and chronic type based upon the dimension of intensity, frequency of exposure and duration of exposure (1). Regardless of their degree of severity, stressor may promote physiological and behavioral disturbances ranging from psychiatric disorders to immune system dysfunction (2).

Exposure to acute stress activates the hypothalamic-pituitary-adrenal axis such that adrenocorticotropin (ACTH) is secreted from the anterior pituitary and acts on the adrenal cortex to release corticosterone in rodents (3). Chronic exposure to stress also alters activity in both pituitary adrenocortical and sympathetic adrenomedullary systems (4).

*Corresponding Author

Differences in the population density is reported to bring about major change in the endocrine responses like increased adrenocortical activity, as revealed by increased adrenal weights, decreased thymus weight and enhanced ACTH-elicited corticosterone release. Crowding was found to decrease body weight and increase adrenal epinephrine secretion, thereby indicating that crowding may be considered as a stressful stimulus (5).

The organism usually responds to stress with a variety of behavioral, biological and cognitive changes. Stressful events may profoundly influence the use of alcohol or other drugs (6, 7). Alcohol consumption can reduce the magnitude of organism's response to stress. This reduction is called stress response dampening effect or tension reduction (8). Prolonged exposure to ethanol concentrations are initially known to stimulate corticosteroid secretions and induce alterations in hypothalamo-pituitary axis that cannot be evinced by shorter exposure (9). Ethanol preference in rats is highly influenced by environmental factors. There is considerable body of evidence showing that housing and social factors influence ethanol intake in rats (10–12). The environmental factors (presumably all of which are not cultural, attitudinal ones) that influence excessive human alcohol intake need to be examined in this perspective using animal models.

The present study proposes to determine the effects of a voluntary and *ad lib* ethanol consumption model, much similar to that of human behavior. We examined the difference in intensity and duration of overcrowding stress on voluntary ethanol intake and ethanol preference in albino rats. The aim of the experiment was to determine the effect of acute and chronic stress situations on free choice ethanol intake in rats.

## METHODS

Animals used in this experiment were adult male albino rats of Wistar strain. The rats were 120 days old and weighing 175–275 g at the beginning of the experiment. The animals had not had any experience in the intake of any other liquids except tap water before the start of the experiments. Rats were maintained on a 12–12 h light-dark cycle under standard laboratory conditions. The animals were housed in groups of two per cage for two weeks to adapt to the novel laboratory conditions.

The control group was small consisted of ten male rats kept under standard laboratory conditions for one week without any stress exposure except for daily handling. The Stressed Groups had sixteen rats divided into two groups of eight rats each and these animals were exposed to overcrowding stress.

(a) *Acute overcrowding stress*: This group of eight rats was kept in a single small polypropylene cage (25 x 20 x 15 cm) so that minimum mobility was possible for the rats inside their cage. These rats were kept in this overcrowded condition daily for six hours only between 9 AM to 3 PM. Acute overcrowding stress was studied for a period of 7 days.

(b) *Chronic overcrowding stress* : Another group of eight rats was kept overcrowded

in a separate cage and these animals were kept in this crowded situation continuously for one week.

The rats in the above three groups had continuous access to two bottle fluid sources. One bottle contained tap water and the other containing 2% w/v alcohol solution, prepared from 95% ethanol mixed with tap water. The positions of the bottles were randomly altered daily to prevent place preference, if any. All the animals had free access to food throughout the duration of the experiment.

Total body weight, food intake and fluid consumption (both water and ethanol) were monitored daily between 10 AM to 12 noon. Daily ethanol intake was calculated in g/kg body weight and expressed as total alcohol intake. Alcohol preference was calculated taking percentage of total fluid intake drunk as alcohol (amount of alcohol consumed/total fluid x 100).

Data are presented as Means ± SD. Comparison between control, acute crowding stress and chronic crowding stress were analysed by Kruskal Wallis Test. Post-Kruskal Wallis multiple comparison criterion was done to determine the level of significance between groups. Wilcoxon Signed Ranks Test was used for the statistical analysis between one day and 7 days. The criterion for statistical significance was $P<0.05$.

## RESULTS

There was a significant ($P<0.001$) decrease in the body weight after one day and 7 days of acute stress. More significant decrease was observed when the animals were kept in the chronic stress situations (one-day, $P<0.05$ and 7 days, $P<0.001$) (Table I). Body weights continued to decrease from first day of stress exposure to 7th day stress, in both acute ($P<0.05$) and chronic stress ($P<0.01$).

Food intake decreased ($P<0.001$) after one-day chronic stress. This decrease in food intake after exposure to chronic crowding for one-day was significantly ($P<0.001$) more than 7 days acute stress, 7 days chronic stress and control groups (Table I). No significant change in the food intake was seen in the acute stress both in one-day and 7 days group compared to control animals.

TABLE I: Effect of acute and chronic stress on body weight, food and fluid intake in rats.

| *Parameters* | *Control* | | *Acute stress* | | *Chronic stress* | |
|---|---|---|---|---|---|---|
| | *1 day N=10* | *7 days N=10* | *1 day N=8* | *7 days N=8* | *1 day N=8* | *7 days N=8* |
| Body weight changes (g) | 0.00±0.00 | –0.60±0.003 | –0.30±0.002 | –11.3±0.02*■■■ | –2.25±0.003■ | –30.5±0.08**■■■ |
| Food intake (g/100 g b.wt) | 7.51±0.66 | 7.18±0.96 | 6.21±0.53 | 6.23±0.42 | 1.83±0.24**■■■ | 7.24±0.71 |
| Water intake (ml/100 g b.wt) | 7.09±1.57 | 13.15±0.18* | 5.90±0.35 | 16.01±0.62** | 6.56±0.73 | 24.26±1.95**■■■ |
| Ethanol intake (ml/100 g b.wt) | 4.67±0.60 | 7.73±0.34* | 6.19±1.25■ | 6.92±0.98 | 6.67±2.11■ | 19.08±1.44**■■■ |
| Total fluid intake (ml/100 g b.wt) | 11.76±1.29 | 20.67±2.38* | 11.87±1.49 | 22.71±2.52* | 12.56±1.37 | 43.48±2.44**■■■ |

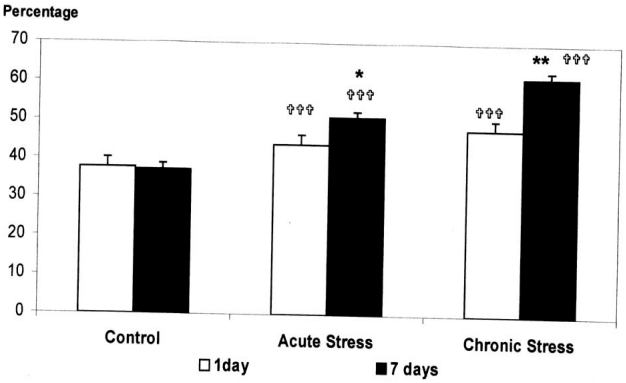
Values are Mean ± SD. N = Number of animals, – decrease.

Significance : *P<0.05, **P<0.01, ***P<0.001 – One-day Vs 7 days (Wilcoxon Signed Ranks Test)
■P<0.05, ■■P<0.01, ■■■P<0.001 – Control Vs Acute stress Vs Chronic stress (Kruskal Wallis Test)

Animals were drinking increased (P<0.01) amount of water after 7 days stress exposure both in acute and chronic stress situations as compared to one-day stress period. Compared to control and acute stressed group, 7 days chronic stressed rats were drinking significantly (P<0.001) more amount of water. No significant difference in the water intake was observed after acute exposure to overcrowding stress compared to control animals (Table I).

Analysis of ethanol intake showed that there was no significant change in the ethanol intake in rats exposed to 7 days acute overcrowding stress compared to control group for the same duration (Table I). One day acute stress exposure made the rats to drink increased (P<0.05) amount of ethanol than the one-day control rats. Chronic crowding for 7 days increased (P<0.001) ethanol intake more significantly than control and acute stress situations. On the other hand, ethanol intake after one-day chronic stress was significantly (P<0.05) more than control as well as acute stress for the same period (Table I). As there was increase in water intake and ethanol intake, from day one to day seven in the control group, there was significant (P<0.05) increase in total fluid intake after 7 days. Similar significant increase in total fluid intake was recorded both in acute (P<0.05) and in chronic stress (P<0.01) exposures (Table I).

Analysis revealed that acute and chronic overcrowding stress increased (P<0.001) the preference for 2% w/v ethanol compared to control. Even after one-day stress, there was substantial increase (P<0.001) in the preference for ethanol compared to control animals (Fig 1). This increased preference was significantly (P<0.001) more after




Significance : *P<0.05, **P<0.01 - One day Vs 7 days

✞✞✞P<0.001 - Control Vs Acute stress Vs Chronic stress

chronic stress for one day. When the stress period was prolonged to 7 days, chronically stressed animals were drinking more than 60% of their total fluid as ethanol (P<0.001), whereas in the acute stressed animals, the preference was 50% (P<0.001). When the ethanol intake in terms of body weight of the animals were analyzed, the rats exposed to chronic crowding stressful situation were

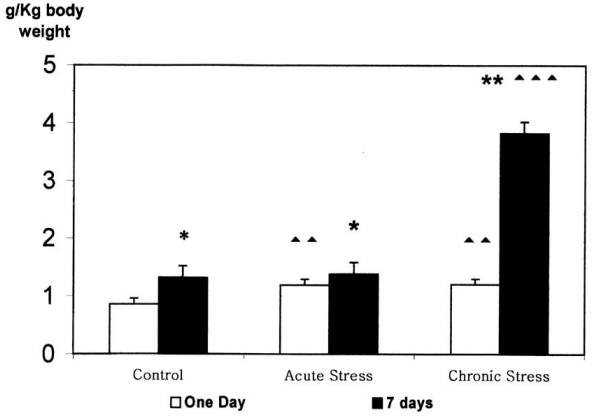



Fig. 2 : Acute and chronic stress and total alcohol intake

Significance : *P<0.05, **P<0.01 - One day Vs 7 days

▲▲P<0.01, ▲▲▲P<0.001 - Control Vs Acute Vs Chronic stress

drinking significantly ($P<0.001$) more ethanol after 7 day stress (3.82 g/kg body weight) compared to acute stress and control for the same duration (Fig 2). Total ethanol intake did not show significant increase in the case of acute exposure to overcrowding condition. But ethanol intake relative to body weight was significantly ($P<0.05$) more after 7 days acute stress than one-day stress. Total ethanol intake after one-day stress was significantly ($P<0.05$) more both in acute and chronic crowding situation than control (Fig. 2).

## DISCUSSION

In the recent past couple of years, studies on effects of animal stress have gained a remarkable interest. Different aspects concerning hypothalamo-pituitary-adrenal (HPA) activity during chronic exposure to various stressors have been repeatedly considered by several authors (13, 14). However, more recently many investigators have considered it necessary to compare the acute response to stress to a chronic response. Animal models of chronic stress are being studied extensively in order to determine the stress-induced changes in neurochemical and neuroendocrine parameters that might be associated with development of human disorders for which stress could be a major contributing factor (15).

Numerous studies have revealed that stress increases ethanol consumption in animals and that individual animals may differ in the amount of ethanol they consume in response to stress (11, 12, 16). In the present work, a more significant increase in the ethanol preference was observed only after chronic crowding stress. There was no increase in the ethanol intake after acute stress (6 hr/day stress) and also after one-day chronic stress in rats. Rats exposed to chronic stress showed increased preference and intake of ethanol compared to acute stressed groups. Because in the chronic stress, rats were exposed to an unavoidable situation, which might have brought about the necessary hormonal changes indicative of the stress response, including increased levels of corticosterone (17). The increased secretion of adrenal corticosteroid might have contributed to the three-fold increase in the ethanol consumption (18). Animal studies reporting a positive correlation between stress and ethanol consumption suggest that drinking of ethanol may take place in response to chronic stress perceived as unavoidable (19, 20).

Water intake and total fluid intake increased approximately two folds after chronic stress for 7 days. The increase in total fluid intake was because of significant increase in the alcohol intake after 7 days. In the present study a decrease in the body weight was observed after crowding stress. Greater the intensity of stress, more the body weight loss was seen. Decreased body weight of the chronically crowded rats in the initial period of exposure to stress might be attributed to lower food intake induced by the high competition for food among these animals in a crowded ambiance. But when the stress period was prolonged to 7 days, there was recovery in the food intake. Hence, the effect of food intake appeared more marked only during the initial period of exposure to the chronic stress. Subsequently, the animals showed signs of partial adaptation characterized by recovery in food intake. But acute stress did not influence

the food intake also as the animals were crowded only for 6 hours per day and even though there may be competition for food intake during this period, the rats compensated the intake during the remaining period of the day when they were not kept under stress.

The observed decrease in food intake after one day chronic stress could be partly due to increased ethanol intake. Ethanol itself may affect digestion and absorption of complex food. Many researchers had reported that ethanol drinking rats exhibit reduced food intake almost in direct proportion to the calories obtained from ethanol (21, 22). But after 7 days of stress, animals showed improvement or recovery in food intake in spite of higher ethanol intake.

The observed decrease in body weight after stress could be due to increased ethanol intake in rats after prolonged stress. Ethanol is known to impair the energy balance in rat and decrease the body weight and body energy (22, 23). Long-term ethanol intake is known to decrease food intake and inhibit growth in experimental animals (24). But our data suggest that chronic stress produced recovery in food intake after 7 days. From the data obtained in this present study, it is evident that as a result of increased ethanol intake, there was marked reduction in body weight both in acute and chronic stress situations without necessarily affecting the food intake. Therefore energy expenditures were increased. This is based on our assumption that the total amount of food intake means same amount of energy assimilated. Moreover, ethanol might increase energy expenditure indirectly by causing restlessness particularly during sleep periods. We cannot ignore the possibility of ethanol affecting digestion and also the role of ethanol and its metabolites in influencing autonomic balance of the organism in an organ specific manner.

Short lasting stressors did not increase alcohol-drinking behavior in rats. This may be due to adaptation or habituation to the stressor, which restore the initially altered physiological response of the animals. From the neurophysiological studies of habituation, it is known that the less intense the stimulus, the more pronounced is the habituation (25). Whereas, there was an increase in the preference for alcohol and marked increase in the alcohol intake in a free choice situation after chronic stress. Animals in the chronic stress state did not show habituation in drinking behaviors. More intense the stressor, the quicker chronic stress develops and this long lasting stressor overburdens the physiological mechanisms eventually leading to behavioral disturbances. Hence, there is increase in voluntary drinking behavior in rats exposed to chronic crowding stress.

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