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Original article

Effect of Deprivation and Stress phases on Voluntary alcohol Intake in Wistar rats through various Behavioral models

Ambareesha. Kondam [a*], M. Chandrashekar[b], Nilesh N. Kate[c], M. Suresh[d], E. Kayalvizhi[e]& M. Geetharani[f]

[a*] *Tutor in Department of Physiology, Meenakshi Medical college & Research institute, Kanchipuram, MAHER University*
[b*] *Professor in Department of Physiology, Meenakshi Medical college & Research institute, Kanchipuram, MAHER University*
[c*] *Assistant Professor in Department of Physiology, Meenakshi Medical college & Research institute, Kanchipuram, MAHER University*
[d*] *Tutor in Department of Physiology, Department of Physiology, Meenakshi Medical college & Research institute, Kanchipuram, MAHER University*
[e] **Assistant Professor in Department of Physiology, Medical college & Research institute, Kanchipuram, MAHER University*
[f] *PG in Department of Physiology, Meenakshi Medical college & Research institute, Kanchipuram, MAHER University*

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ABSTRACT

Alcohol intake in several animals including man depends on numerous genetic and environmental factors. One of the major factors that can affect alcohol intake is Alcohol Deprivation Effect (ADE). Another factor that also can have same effect on voluntary alcohol intake is stress. Stress has long been thought to influence the initiation and maintenance of alcohol drinking in humans. For the alcohol drinking procedure, a long-term model of alcohol self-administration originally developed for Wistar rats was used and was compared with different ethanol concentration along with behavioral assessments. **Methods:** The experiment was carried out with 24 healthy Wistar rats. Proper Animal Ethical permission was obtained from IAEC & CPCSEA before the commencement of animal experiment. The animals were randomly divided into four groups of 6 animals each. Group – I (non alcoholic): Control Group, which consists of 6 animals were randomly divided into 3 sub groups of 2 animals. Each sub group was given only tap water, then after 3 weeks, behavioral assessment was done by open field apparatus for central Peripheral ambulation, grooming, rearing and immobilization. Group – II (alcoholic group) consists of alcohol fed 6 animals were randomly divided into 3 sub groups of 2 animals. Each sub group was given tap water and 5%, 20% & 40%, ethyl alcohol solution about 3 weeks. Group – III, it is also alcoholic group consists of alcohol fed 6 animals were randomly divided into 3 sub groups of 2 animals. Each sub group was given tap water and 5%, 20% & 40%, ethyl alcohol solution about 3 weeks. Stress was applied after deprivation and the consumption of alcohol was assessed. Group – IV, it is a Preference group consists of 6 animals, were randomly divided into 3 sub groups of 2 animals. Each sub group was given tap water and 5%, 20% & 40%, ethyl alcohol solution for about 3 weeks. The behavior was assessed before and after stress effect. **Results:** ADE led to a significant increase ($p<0.05$) in alcohol intake in Wister rats. After deprivation, however, their preference for the 20% alcohol solution was increased. Repeated swim stress caused an increase in alcohol intake and they responded to behavioral changes before and after ADE and stress. **Conclusion:** This study reveals the effect of alcohol deprivation and stress on the drinking behavior at different concentration with specific or non specific preference groups with its behavioral modulations. Wister rats differentially respond to alcohol deprivation and stress. In these animals alcohol drinking is an attempt to cope with stress.

1. Introduction

Alcohol intake by several animals including man depends on number of genetic and environmental factors [1]. One of the major factors that can affect alcohol intake is ADE (Alcohol Deprivation Effect) [2]. Another factor that also can have same effect on voluntary alcohol intake is stress [3]. The term 'stress' is often used to describe the subjective feeling of pressure or tension.[4] . Stress has long been thought to influence the initiation and maintenance of alcohol drinking in humans [5].. The young adolescent brain shows higher sensitivity to alcohol induced brain damage and

* Corresponding Author : Ambareesha. Kondam
Tutor in Department of Physiology,
Meenakshi Medical college & Research institute,
Kanchipuram, MAHER University
Email:ambreesh.kondam@gmail.com

cognitive impairment [6]. Furthermore, the onset of alcohol use during adolescence may have potentially long-lasting consequences. That could lead to a greater risk for developing alcohol dependence in adulthood [7].

2.Materials and Methods

2.1.Animals: The present study was conducted at Department of Physiology MMC & RI, Enathur, and Kanchipuram. The experiment was carried out with 24 healthy Wistar rats. Then animals were randomly divided into four groups of 6 animals each. Deprivation pattern in all groups was assessed with open field apparatus. The last two groups were stressed by forced swim test special plastic tanks.

Wistar albino rats (200±20g) were procured from Kings Institute Guindy (Chennai). The animals had free access to standard rat pellet with watch water supplied adlibitum and strict hygienic conditions. Animals were habituated to laboratory conditions (i.e. room temperature of 25±2C; relative humidity 45-55% and a 12:12 light/dark cycle) for 48h prior to experimental protocol to minimize non specific stress if any. The approval of the Institutional Animal Ethical Committee (IAEC) of MAHER was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the purchase of control and supervision of experiments on animals (CPCSEA).

2.2.Experimental design

The experiment was carried out for a period of 75 days with 24 healthy albino rats. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for one week. Then animals were randomly divided into four groups of 6 animals each.

2.3. Alcohol self administration procedure

After 2 weeks of habituation in the animal room, rats were given ad libitum access to tap water with 5%, 20% & 40% ethanol solution along with food. Alcohol drinking solutions were taken from 99.9% ethanol diluted with tap water to the different concentrations. Spilling and evaporations were minimized by the use of special bottle caps. With this procedure the ethanol concentration in a given solution stayed at least 1 week. The position of the bottles was changed weekly to avoid location preferences. In the present study the first group was taken as a control treated with tap water. Second and third groups were divided into three consecutive subgroups. Each sub group received 5%, 20% and 40%ethonol along with tap water. Fourth group was kept as preference group.

2.4.Alcohol deprivation procedure

All rats underwent a 2 week deprivation cycle and 8 weeks of continuous alcohol availability. After the deprivation period, rats were given access to alcohol again for the rest of the experiment. To measure the alcohol deprivation effect, alcohol consumption was measured daily until a stable base line was established. After the deprivation phases, alcohol intake and the preference were measured daily for 4 days.

2.5.Stress induced alcohol intake

A stable baseline consumption of alcohol was established before the stress procedures were applied. The test procedure was performed for 10min for 3 consecutive days. Then alcohol consumption was measured, daily before each stress procedure and 2 days after the stress procedure. To perform the forced swim test, rats were placed into cylindrical plastic tanks (55cm height and 35cm diameter). Filled with tap water up to a level o f 40 cm. The animals were observed for the latency time until the animal started to float was measured. After the swim stress, animals were dried with a towel and returned to their home cages.

2.6.Alcohol deprivation and stress induced behavioral changes

The rodent behavior changes were observed by using open field apparatus.

2.7.Behavioral study- by Open field test

This test is performed in the control group of Wister rats and the reading for ambulation, rearing, excreting feces, and urine and whether it is mobile or immobile were assessed. One week after the completion of the voluntary alcohol drinking, the rats were used in the open field apparatus consisted of four identical controlled cages. Each cage was transected by two perpendicular, co-planar arrays of 16 infrared photocells which were intended to measure forward locomotion by determining the rats position every 0.1s. The forward locomotion was defined as the distance in 9(inch) travel by the rat during the five minutes test session. Another set of photo cells located 15 cm above the cage floor measured the number of rearing. After initial habituation of 5 min to the test room each rat was introduced to the test cage for another 5 min. The cages were cleaned carefully between the recordings. The test sessions were conducted between 10.00 and 14.00 to avoid errors attributed to the variations in motor activity of the day activity cycle.

2.8.Statistics

The statistical package SPSS (17.0 VERSION) was used. Data obtained from alcohol deprivation and stress experiments behavioral measures were analyzed by using two-way analysis of variance (ANOVA) with repeated measures. $P< 0.05$ had taken as statistically significant.

3.Results

All the three groups (except Group one) showed an increase in total alcohol intake during the first 4 weeks of the acquisition period with the increase consumption mainly from the 5% solution. Thereafter all 3 alcohol fed Groups animals maintained a stable level of basal total alcohol intake and preference over the next 4 week before deprivation.

After alcohol deprivation, all 3 alcohol Group animal lines slightly increased basal total alcohol intake, but total alcohol preference stayed stable over the whole time course of the experiment. For intake and preference, respectively the following analysis showed that alcohol deprivation led to a significant increase in alcohol intake and preference in unselected Wistar rats after deprivation on days 1 -14.The total alcohol preference increased temporarily in group 2, 3 & 4 animals from 1.0 ± 0.3 before deprivation to 1.9 ± 0.2 on the first day after deprivation and

was still significantly elevated on the fourteenth day 1.9 ± 0.3 but not on the fifth day or later. For precluding any increases being caused by deprivation, a stable baseline of ethanol consumption was established before all rats underwent a deprivation phase of 2 weeks after 8 weeks of alcohol access leaving animals with food and water ad libitum. After the deprivation period, alcohol solutions were returned and alcohol consumption was measured daily for the following 4 days.

GROUP -I (Control Group / non alcoholic)

water n2	water n2	water n2	ADE 2weeks	Behavioral assessment

Group – II (non stress alcoholic)

5% water	20% water	40% water	ADE 2weeks + Behavioral assessment	Alcohol intake after ADE	Behavioral assessment

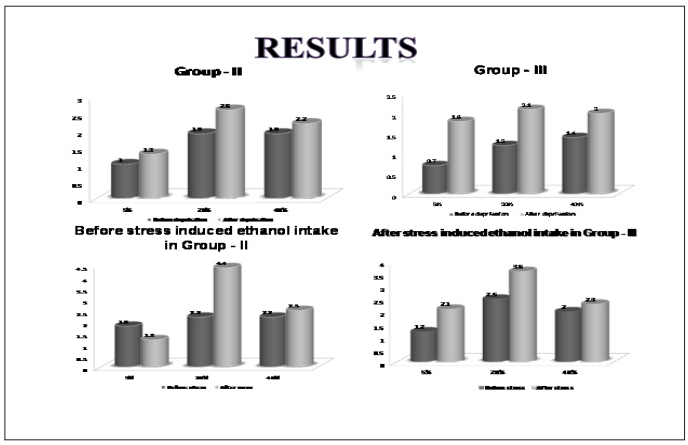
Group – III (stressed alcoholic)

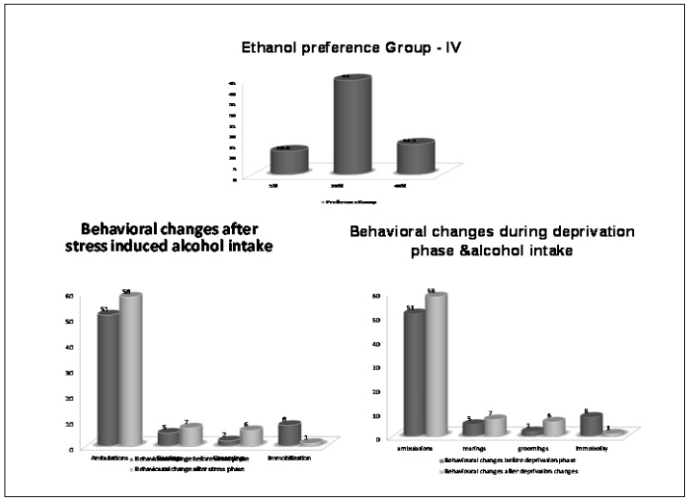
5% water	20% water	40% water	ADE 2weeks	Stress by forced swim test	Alcohol intake after stress	Behavioral assessment

Group – IV (Preference group):

A 2-animals			B 2-animals			C 2-animals			ADE 2weeks	Behavior	Stress	Behavior
5% water	20% water	40% water	5% water	20% water	40% water	5% water	20% water	40% water				

Note - 5%- alcohol, 20%- alcohol, 40%- alcohol.





Alcohol deprivation effect (ADE) after 2 weeks of alcohol deprivation, we could observe a significant increase in alcohol intake in all groups except non alcoholic group indicating the occurrence of an ADE. Animals increased their ethanol intake after the withdrawal phase for more than two folds that declined to the baseline level after the 14th or15th post deprivation day.

Clinical observations suggest that stimuli associated with the availability or consumption of ethanol can evoke subjective feelings of craving and trigger episodes of relapse in abstinent alcoholics [8].Changes in alcohol intake caused by forced swim stress for 10 min on 3 consecutive days were significantly different between the four rat groups. The preference values for the selected lines before deprivation were higher than normal, thus precluding any increases being caused by stress. Post hoc comparisons revealed a significant increase in alcohol consumption by unselected Wistar rats after swim stress for 5min on 3 consecutive days led to a significant increase in alcohol consumption in Wistar rats. In particular, intake from the 20% alcohol solution was increased after forced swim stress (2.6 ± 0.2).

The values that were analyzed shows that the animal behavioral parameters such as ambulation, rearings and grooming were increased in comparison with normal phase ethanol intake. Behavioral changes were observed after stress phase of alcohol intake by using open field apparatus. The data analyzing that during stress phase, the animal behavioral parameters such as ambulations, rearings and grooming were decreased in comparison with normal phase ethanol intake.

4. Discussion

Stressful life events influence alcohol drinking and relapse behavior. In some individuals alcohol drinking is an attempt to cope with stress, the stress induced alcohol drinking has a significant genetic component [9]. Increase in 20% solution intake shows that exposure to stress can influence the ingestion of alcohol, but its effect is stressor specific [10]. The ADE observed in animals reflects some aspect of alcohol relapse in humans [11].

In the present study, a rat model of long-term alcohol-drinking and alcohol deprivation is described, which mimics relapse behavior in human alcoholics. Long-term voluntary alcohol drinking with repeated alcohol deprivation episodes has been suggested as animal model for some aspects of alcoholism. Alcohol deprivation induced hyper locomotion in both groups.. However, alcohol intake of the repeated alcohol deprivation group was significantly increased compared to the first alcohol deprivation group at the end of the experiment. It is concluded that repeated alcohol deprivation experience might promote the development of alcohol addiction because of its latent stimulating effect on alcohol drinking that can be unveiled by (presumably mildly stressful) experimental situations.

The long-term goals of this proposal are for the better understanding the neurobiological mechanism involved in alcohol relapse. The goals of this application are to (a) better characterized the long-term "Alcohol Deprivation Effect" (ADE) that develops in some experimental animals following prolonged (1 week or longer) alcohol abstinence and (b) determine if any neuroadaptive changes in certain monoamine systems are associated with the ADE. The ADE is described as an enhanced intake of ethanol following a period of alcohol deprivation. The following chronic alcohol drinking and extended abstinence, long-term neuroadaptations, involving alterations in certain serotonin (5-HT) pathways, develop in individuals at risk for alcoholism, which maintain or enhance the rewarding effects of ethanol and contribute to the appearance of the ADE and alcohol relapse. This hypothesis using adult male alley demonstrate an ADE following an alcohol deprivation period of one week or longer. Furthermore, if the ADE observed in animals reflects some aspects of alcohol relapse in humans, then the findings from this application may provide some fundamental knowledge of possible neurobiological factors contributing to alcohol relapse. However, in this study, long-term alcohol drinking behavior was comparatively studied in unselected Wistar rats. Furthermore, the effects of alcohol deprivation and stress were examined in all animals. After an alcohol deprivation phase of two weeks, a significant transient increase in voluntary alcohol intake and preference (ADE) ensued in the Wistar rats [12]. Repeated swim stress caused a slight increase in alcohol intake in Wistar rats.

Thus, alcohol consumption and preference were significantly enhanced after ADE effect of the alcohol solutions. Animals increased their alcohol intake and preference approximately two fold on the first day of the ADE. This increased alcohol intake declined to baseline drinking levels by the fifth re exposure day. In agreement with previous findings, a detailed analysis of intake for 5%, 20% & 40% alcohol solutions showed a preference shift toward the highly concentrated 20% alcohol solution during the ADE. Alcohol consumption after short (24 hr) deprivation phases but only for the first hour of renewed access to alcohol [13]. After alcohol deprivation, unselected Wistar rats showed a very pronounced ADE after repeated deprivation phases. Interestingly, rather than the temporary increase after deprivation usually seen with the ADE. This might be true for besides alcohol deprivation, stressful life events and maladaptive responses to stress influence alcohol drinking and relapse behavior. Although the relationship between stress and alcohol drinking in humans [14] and laboratory animals [15] is complex, it might be that in some individual's alcohol drinking is an attempt to cope with stress.

In the present study, the effect of stress on alcohol drinking was examined in unselected Wistar rats groups because it is known that stress-induced alcohol drinking has a significant genetic component [16]. Wistar rats after being exposed to swim stress for 3 consecutive days temporarily increased their alcohol intake after the last stress day. Well, but the possibility cannot be excluded that the increase in 20% solution intake that they showed after deprivation was only a continuation of the increasing trend starting before deprivation; however, only a small but significant effect was observed in Wistar. These findings show that exposure to stress can influence the ingestion of alcohol, but this effect is stressor specific and has a large inter individual variability.

Thus, if both stressors produce different physiologic and hormonal consequences for a stressed subject, it is not surprising that the effect of stress on ingestion of alcohol is stressor specific. At the end applied stress was observed after the consumption of alcohol by the animal which increase behavioral stress. It should be pointed out that the sequence of alcohol deprivation, swim stress was always the same.

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