DOI: *http://dx.doi.org/10.22122/ahj.v10i3.577* *Published by Vesnu Publication*

The Effect of Chronic Ethanol Consumption on Sexual Motivation and Behavior of Adult Male Wistar Rats in the Copulatory Phase

Maryam Sadeghzadeh[1], Alireza Shirpoor PhD[2], Naser Khalaji PhD[3], Roya Naderi PhD[4], Mahrokh Samadi[1], Yousef Rasmi PhD[5]

Original Article

Abstract

Background: The interaction of ethanol consumption and sexual behavior has been evaluated over the past three decades; however, some studies have assessed how ethanol consumption affects the general behavioral aspects of the copulatory cycle patterns in male rats. The aim of this study was to investigate the effect of chronic ethanol consumption on adult male Wistar rats' sexual motivation and behavior alteration in pre-copulatory, copulatory, and executive phases of the copulatory cycle.

Methods: Male Wistar rats were randomly allocated to two groups (control and ethanol treated groups). After 42 days of treatment, male rats were given access to adult female rats for 2 hours and their sexual behavior were recorded in a fully dark room using an infrared camera.

Findings: Chronic ethanol consumption caused a significant increase in anogenital sniffing and mounting, intermission, and ejaculation latencies periods, as well as a significant decrease in the sexual activity index (SAI) and copulatory efficiency (CE) compared to the control group.

Conclusion: It is suggested that chronic ethanol consumption suppresses sexual behavior and reduces male rats' tendency toward sexual interaction with female rats as manifested by the enhanced latency periods in the copulatory phases and reduced SAI of ethanol treated animals.

Keywords: Ethanol; Sexual behavior; Rats; Sexual motivation

Citation: Sadeghzadeh M, Shirpoor A, Khalaji N, Naderi R, Samadi M, Rasmi Y. **The Effect of Chronic Ethanol Consumption on Sexual Motivation and Behavior of Adult Male Wistar Rats in the Copulatory Phase.** Addict Health 2018; 10(3): 190-7.

Received: 15.03.2018 **Accepted:** 09.05.2018

1- MSc Student, Department of Physiology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
2- Professor, Nephrology and Kidney Transplant Research Center, Urmia University of Medical Sciences, Urmia, Iran
3- Associate Professor, Department of Physiology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
4- Assistant Professor, Department of Physiology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
5- Professor, Department of Biochemistry, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
Correspondence to: Alireza Shirpoor PhD, Email: ashirpoor@yahoo.com

Introduction

Due to the higher prevalence of alcoholism among men, as opposed to women, the interaction of alcohol consumption and male sexual behavior has become of interest. Consequently, a great many studies have been conducted to elucidate the effect of ethanol consumption on male sexual dysfunctions and sexual behavior.[1-3] Several lines of evidence indicated that ingestion of ethanol results in a variety of structural changes in the reproductive system such as alteration in spermatogenesis and sperm motility, reduction of male reproductive organ weight, reduction of circulating testosterone levels, as well as impaired epididymal sperm maturation, and reduction of cauda epididymal sperm content.[4-6] The association between ethanol consumption and sexual behavioral alteration is ambiguous.[7] Contrary results based on drinking patterns, age, genesis of the animal, and dose of ethanol have been reported in previous studies. Regarding the dose of ethanol, Cagiano et al. have reported that prolonged low-dose ethanol intake does not significantly affect the copulatory activity of male rats including mount-intermission latency and frequency, ejaculation latency, and post-ejaculatory interval.[8]

In contrast, other studies have shown that low to moderate doses of ethanol consumption by male rats led to an increase in latencies including mount, intromission, and ejaculatory latencies.[9] Moreover, the number of intromissions and ejaculations when introduced to a receptive female were affected.[10] Other important impacts of the binge-like pattern of administration of ethanol for 4 weeks on sexual behavior in young adults and adult animals have been showed by Harding et al.[11] According to their report, males undergoing ethanol consumption during adolescence and young adulthood periods showed significantly longer latencies (mount, intromission, and ejaculation latencies) in sexual performance tests compared to the controls.[11] Among adult animals, however, all latencies were more modest, and the long-term effects did not reach statistical significance compared to the control animals.[11]

One of the earliest experimental studies on the effect of ethanol on copulatory behavior has been conducted by Dewsbury.[12] Using a 10% ethanol solution, Dewsbury orally administered ethanol to male rats. The results of the study revealed that ethanol ingestion caused significantly longer mount, intermission, and ejaculation latencies compared to those in the control rats. In addition, ethanol consumption prolonged the mean pause between successive intromissions and lengthened the post-ejaculatory refractory period following ejaculation.[12]

The controversy surrounding the role of alcohol consumption in male sexual behavior as mentioned above tempted us to re-evaluate the subject with the following details. Commonly, previous experiments have evaluated alcohol-induced sexual behavior alterations in male rats when introduced to estrous female (receptive female) rats. In general, the initial behavior of a male rat when meeting a female is the olfactory and visual behavior to find out whether the female rat is in the estrous period or not.[13] Therefore, one goal of the current experiment was to evaluate the effect of chronic and heavy consumption of ethanol on male rats' behavior when introduced to intact females (not obvious whether the females are in the estrous period or not) and compare the effort made by ethanol-consuming rats with control group rats towards detecting estrous females. The second major impetus for this study was to evaluate the effect of chronic ethanol consumption on sexual behavior in the copulatory cycle including the pre-copulatory phase, copulatory phase, and executive phase with details of events such as duration and frequency of events in each phase and compare them with those in the control rats.

Methods

Animals and treatments: All experimental protocols were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication, no.85-23, revised 1985) and were supported by the Animal Care Committee of Urmia University of Medical Sciences, Iran. The experiments were conducted on 20 male Wistar rats (age: 4 months; weight: 280 ± 20 g). The rats were assigned to the control group, and ethanol treated group (n = 10/group). Similar to our previous study, rats in the ethanol group received ethanol with a dose of 4.5 g/kg body weight (Merck KGaA, Darmstadt, Germany) (tap water as

a solvent, 20% w/v) by gavage daily for 6 weeks. The control group was gavaged with tap water.

Sexual behavior testing: At the end of the experiment, sexual behavior of each male rat was evaluated through the follow procedure. The rats were placed in polypropylene shoebox cages for 5 minutes, then, introduced to the female rats in a dark room as habituation. Female rats were also adapted to the same schedule. During this habituation period, male and female rats were allowed to discover the cage in the absence of the opposite sex. After 5 minutes of familiarization to the cage, the female was entered into the cage and sexual measurements were taken. Each male rat was tested for sexual behavior with one intact female in the fully dark room. Moreover, their sexual behaviors were recorded using an infrared camera connected to a computer and saved in Snazzi*4 software (China) for 2 hours. At the end of 6 weeks, recorded videos were replayed and analyzed using GOM player software (in slow motion when necessary) and the following parameters were measured.

Criteria for determining estrous and non-estrous females were the behavior of male and female rats explained below. Generally, male rats in the first contact with females, attempt to detect whether the female is in estrous or non-estrous period by anovaginal sniffing.[14] This is because some pheromones are secreted by estrous females. If the male detects the estrous period in the female, it shows sexual behavior such as mounting, intromission, ejaculation, and etcetera. If the male detects that the female is in non-estrous period after anovaginal investigation, it displays no additional sexual behavior and undergoes a pause situation.[14] In addition, behaviors of estrous and non-estrous females in response to male anovaginal investigations are different. To draw the male's attention, the female in the estrous period displays specific behavior such as hopping (short jumps with all four legs off of the ground) and darting (short and sudden runaway movements in which she presents her body to the male).[14]

As mentioned in the introduction, the first aim of the investigation was the comparison of reorganization attempts of male rats in detecting estrous (receptive) females from non-estrous females. In this case, behaviors such as mouth and anogenital sniffing times and related latencies, as well as pause time of male rats after detecting that the females are in non-estrous period were analyzed and the two groups were compared. Measures of the sexual behavior of males in response to females in estrous period included the following:

1. Mount latency: time from encountering the female rat until the first mount;
2. Intromission latency: time from introduction to the female until the first intromission;
3. Latency to first behavior: time from introduction to the female until the first behavior, that is mount or intromission;
4. Number of mounts;
5. Number of intromissions;
6. Number of ejaculations (if a test is utilized that allows for observation of multiple ejaculation series);
7. Ejaculation latency: time from the first intromission to ejaculation;
8. Post-ejaculatory interval: time from ejaculation until the next mount or intromission (time period before the next intromission);
9. Intromission ratio: the number of intromissions classified by the sum of the number of intromissions and the number of mounts;
10. inter-intromission interval: the total test time classified by the number of intromissions, or the ejaculation latency divided by the number of intromissions;
11. Copulatory rate: the sum of the number of mounts and the number of intromissions divided by the time between the first behavior and ejaculation;
12. Mount frequency (MF): the number of mounts prior to ejaculatory behavior;
13. Intromission frequency (IF): the number of intromissions before ejaculatory behavior;
14. Copulatory efficiency (CE): the number of intromissions classified by the total number of mounts with and without penile insertion;
15. The sex activity index (SAI): calculated by the method proposed by Babaei Balderlou and Khazali[15] as follows:

$$SAI = \log (1/ML \times t) + \log (1/IL \times t) + \log (1/EL) \times t) \sqrt{NM + NI} + Y$$

Normal distribution of data within each group was verified by carrying out a Kolmogorov-Smirnov test. The statistical differences between the groups were determined through Independent-samples t-test. The data obtained from each test are presented as mean ± SE, and $P < 0.05$ is considered as statistically significant.

Results

Chronic exposure to ethanol resulted in alterations in sexual behavior of male animals following introduction to estrous and non-estrous females. Recorded video analyses revealed that 5 out of 10 female rats introduced to the control males were in estrous period and 5 were in the non-estrous state. Among females introduced to the ethanol treated males, 6 females were in estrous period and 4 were in non-estrous period. Based on the mentioned cues, the behavior of the male rats when introduced to the non-estrous female was as described in the following. The anovaginal latency (time lag between introduction to the female and the first anovaginal sniffing) in the ethanol treated males was significantly longer than that in the control males ($P < 0.05$) (Figure 1).

The duration of anovaginal investigation in the ethanol treated group was also significantly longer than that in the control group ($P < 0.05$). It was verified that ethanol administration contributed to a longer investigation effort by rats for detecting whether females were in the estrous condition or not. The data depicting the various sexual behavioral parameters of males when introduced to estrous females are presented in figure 1. Mounting, intromission, and ejaculation latencies were significantly longer in the ethanol treated group in comparison with the control group ($P < 0.05$). The number of mounts and intromissions were not significantly different between the two groups. However, the number of ejaculations was significantly less in the ethanol treated male rats ($P < 0.05$). Intromission rates and mount and intromission frequencies in the ethanol treated males were significantly higher compared to those in the control males ($P < 0.05$) (Table 1). Ethanol consumption significantly reduced CE in ethanol treated rats compared to that in the control rats ($P < 0.05$).

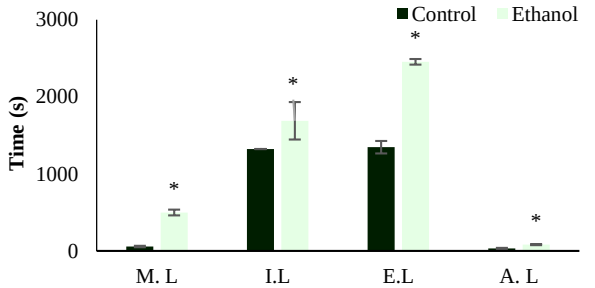

Figure 1. Effect of ethanol consumption on mount latency, intromission latency, ejaculation latency, and anovaginal latency
Values are presented as mean ± SE for 10 rats per group
ML: Mount Latency; IL: Intromission latency; EL: Ejaculation latency; AL: Anovaginal latency
*Denotes significant difference compared to the control

Post-ejaculatory and inter-intromission intervals were significantly higher in the ethanol treated group compared to those in the control group ($P < 0.05$) (Figure 2).

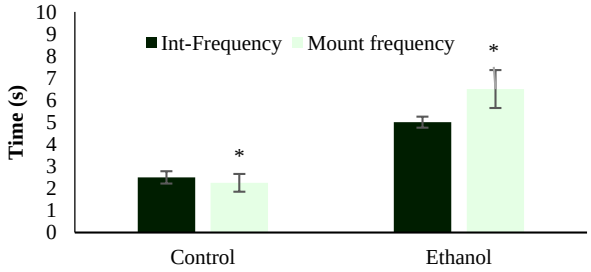

Figure 2. Effect of ethanol consumption on intromission frequency (IF) and mount frequency (MF)
Values are presented as mean ± SE for 10 rats per group.
*Denotes significant difference compared to the control

Table 1. Parameters of male sexual behavior

Parameters	Groups	
	Control	Ethanol
Mount Number	16.50 ± 0.64	20.25 ± 2.70
Int. Number	18.50 ± 0.64	19.25 ± 2.70
E. Number	8.0 ± 0.4	4.70 ± 0.47*
LFB (S)	58.00 ± 9.33	498.0 ± 37.2*
PEI (S)	1142.2 ± 74.9	1518.50 ± 82.48*
Intromission Rate	0.520 ± 0.002	0.570 ± 0.008*
Inter II (S)	72.58 ± 1.88	133.54 ± 14.52*
Capulatory rate	0.0120 ± 0.0002	0.0072 ± 0.0004
CE	1.110 ± 0.006	0.91 ± 0.02*
Pause	162.5 ± 11.9	725.25 ± 261.00*

Values are presented as mean ± SE for 10 rats per group.
E Number: Ejaculation number; LFB: Latency to first behavior; PEI: Post-ejaculatory interval; Inter II: inter-intromission interval; CE: Copulatory efficiency; *Denotes significant difference compared to the control.

Figure 3 shows that the SAI significantly declined in the ethanol treated males compared to that in the control males ($P < 0.05$).

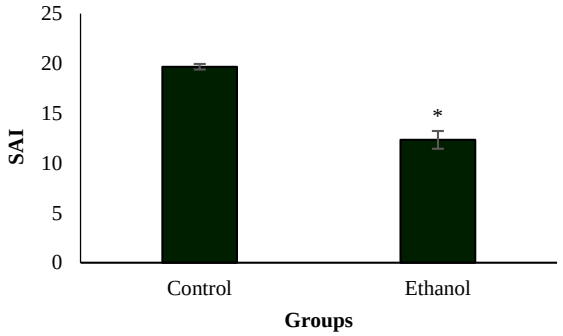

Figure 3. Effect of ethanol consumption on the sex activity index (SAI)
Values are presented as mean ± SE for 10 rats per group.
*Denotes significant difference compared to the control

Discussion

The results of the present study showed that chronic administration of 4.5 g/kg body weight (BW) ethanol induced a reliable increase in the number of level changes in the copulatory behavior of male rats. The results provide a further characterization of behavioral alterations exhibited by rats in the model of chronic exposure to ethanol as reported by previous studies.[16-18] Regarding the first aim of the current study, which was recognizing the behavior of male rats when introduced to intact females, anovaginal latency was significantly longer in ethanol treated males than the control male rats. In fact, sexual motivation lasted for a significantly longer time in rats chronically exposed to ethanol compared to the control males. This suggests that ethanol consumption leads to the inability of male rats to recognize cues indicating sexual receptivity. In contrast to our results, results of the study conducted by Ferraro and Kiefer showed that ethanol exposure led to fewer anovaginal investigations in ethanol treated male rats compared to the control rats.[16] It is possible that investigation length in ethanol treated rats be due to the deleterious effect of ethanol on the olfactory system. This is because besides the role of auditory and visual cues in sexual behavior, olfactory cues play a particularly important role in anovaginal sniffing and distinguishing of receptive females from non-receptive ones.[17] The second issue addressed in this study was the effect of prolonged administration of alcohol on behavioral components of male rats displayed in the copulatory cycle in reaction to estrous females, which was found to be significant. Mounting, intromission, and ejaculation latencies were significantly longer in the ethanol treated group compared to the control group. The number of mounts and intromissions did not differ; however, the number of ejaculations was reduced in the ethanol treated rats.

Nevertheless, in another study, a significant reduction was observed in the number of ejaculations and a rise in initial latency, latency of ejaculation, number of mounts, no changes was observed in the intromissions and latency of intromission following chronic ethanol treatment.[19] In contrast, the results of another study indicated that different doses of ethanol did not cause a significant change in some measures of rats' sexual behavior including mount, intromission, and ejaculation latencies, as well as mount and intromission frequencies compared to the animals receiving saline.[16] Latencies are usually considered in the evaluation of male sexual incentives characterized by a harmonization of sexual desire occurring in the brain and its transmission to the periphery, resulting in penile erection.[20,21] The increased mount and intromission latencies in the ethanol treated group suggest a decreased motivational state, for mount latency is an indicator of sexual motivation.[22] Some plausible mechanisms, through which ethanol exerts its effect on sexual behavior alterations, have been postulated by previous studies.

Several lines of evidence revealed that ethanol consumption directly influenced the hypothalamic-pituitary axis, and consequently, reduced the activity of steroidogenic enzymes, luteinizing hormone (LH), and testosterone levels.[23-25] In addition, ethanol impaired the binding of LH to its receptors on leydig cells surface, suppressed the steroidogenic activity of leydig cells, and decreased the secretion of testosterone by these cells.[26] A large body of accumulated data showed that LH stimulated the regions of the brain that have a role in regulating the male sexual behavior directly and indirectly.[27,28] Moreover, numerous neurochemical mechanisms, such as GABA and dopamine, lesions of medial preoptic area neurons, and the depressant effect of ethanol on

the central nervous system may contribute to sexual dysfunction.[16,29-31]

In the current study, chronic ethanol administration resulted in longer post-ejaculatory intervals (PEI) or longer latencies in initiating behavior after ejaculation (reflected in the PEI) compared to the controls. Contradictory results have been reported about the effect of ethanol exposure on PEI in previous studies. Young rats exposed to ethanol displayed longer PEI, but adult rats exposed to ethanol did not undergo delayed impairment in PEI.[11] Although, the mechanism through which ethanol affects PEI is not well known, there are treatments such as painful electric shock to the skin and midbrain lesions that affect PEI in rats.[32,33] Furthermore, it is established that the reduced excitability in the spinal cord control of penile reflexes has no effect on PEI.[34] Considering all the above, it can be supposed that ethanol-induced alterations in the PEI are due to the effect of some sort of inhibition within the brain. The present study results also showed that ethanol administration results in a significant enhancement in intromission rate, inter-intromission interval, and mount and intromission frequencies, and a significant reduction in copulatory rate compared to control rats. The inter-intromission intervals and copulatory rates are particularly important indicators and are often interpreted as measures of temporal patterning of copulation.[14] Thus, it can be proposed that the decrease in the number of ejaculations and copulatory rates after ethanol consumption is compensated by an increase in the intromission rate, inter-intromission interval, and mount and intromission frequencies. Another important finding of this study was the significant decrease in CE and SAI in the ethanol group as compared to the control group. The decreased level of CE and SAI in the ethanol treated animals provides strong evidence confirming the suppressive effect of ethanol on reproductive behavior in male rats. Finally, the results of the present study revealed all the potentials and pitfalls of different paradigms of rats' sexual behavior.

Conclusion

Our results suggest that chronic ethanol exposure showed a reduction in sexual motivation and sexual desire as well as sexual receptivity and performance in males, suggesting that exposure to ethanol can negatively affect the various sexual behavior parameters of the male rats. Further research, nevertheless, is required to elucidate the mechanisms through which ethanol produces these behavioral deficits in order to develop practicable prevention or treatment strategies for alcohol induced adverse effects in the reproductive system.

Conflict of Interests

The Authors have no conflict of interest.

Acknowledgements

This work was derived from a Master of Science thesis in physiology in Urmia University of Medical Sciences, Urmia, Iran. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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DOI: *http://dx.doi.org/10.22122/ahj.v10i3.577* *Published by Vesnu Publication*

تأثیر مصرف مزمن اتانول بر تحریک جنسی و رفتار جنسی موش‌های صحرایی بالغ نر نژاد ویستار در دوره جفت‌گیری

مریم صادق‌زاده[۱]، دکتر علیرضا شیرپور[۲]، دکتر ناصر خلجی[۳]، دکتر رویا نادری[۴]، ماهرخ صمدی[۱]، دکتر یوسف رسمی[۵]

مقاله پژوهشی

چکیده

مقدمه: با وجود این که اثرات مصرف اتانول و رفتار جنسی در سه دهه گذشته مورد توجه قرار گرفته، اما مطالعات اندکی به بررسی چگونگی تأثیر مصرف آن بر جنبه‌های عمومی رفتاری در الگوی سیکل جنسی موش‌های صحرایی نر پرداخته است. هدف از انجام پژوهش حاضر، بررسی اثر مصرف مزمن اتانول بر تحریک جنسی و تغییرات رفتاری در مراحل قبل از جفت‌گیری، جفت‌گیری و بعد از جفت‌گیری بود.

روش‌ها: موش‌های صحرایی نر بالغ به دو گروه شاهد و مصرف‌کننده اتانول تقسیم شدند. پس از ۴۲ روز دریافت اتانول، موش‌های صحرایی نر به مدت دو ساعت در مواجهه با موش‌های صحرایی ماده قرار گرفتند و رفتار جنسی آن‌ها در یک اتاق تاریک با استفاده از دوربین مادون قرمز ثبت گردید.

یافته‌ها: مصرف مزمن اتانول، سبب تأخیر معنی‌دار در دوره‌های جنسی شامل بوییدن دهان و واژن، سوار شدن بر پشت، دخول و انزال و همچنین، کاهش معنی‌دار شاخص فعالیت جنسی و جفت‌گیری مؤثر نسبت به گروه شاهد شد.

نتیجه‌گیری: بر اساس نتایج به دست آمده، مصرف مزمن اتانول منجر به تضعیف رفتار جنسی و کاهش تمایل موش صحرایی نر برای فعالیت جنسی می‌شود که در مطالعه حاضر این مسأله به صورت افزایش دوره‌های تأخیری در مرحله جنسی و کاهش شاخص فعالیت جنسی در حیوانات الکلی نشان داده شد.

واژگان کلیدی: الکل، رفتار جنسی، موش‌های صحرایی، دوره جنسی

ارجاع: صادق‌زاده مریم، شیرپور علیرضا، خلجی ناصر، نادری رویا، صمدی ماهرخ، رسمی یوسف. **تأثیر مصرف مزمن اتانول بر تحریک جنسی و رفتار جنسی موش‌های صحرایی بالغ نر نژاد ویستار در دوره جفت‌گیری.** مجله اعتیاد و سلامت ۱۳۹۷؛ ۱۰ (۳): ۱۹۷-۱۹۰.

تاریخ دریافت: ۹۶/۱۲/۲۴ **تاریخ پذیرش:** ۹۷/۲/۱۹

۱- دانشجوی کارشناسی ارشد، گروه فیزیولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی ارومیه، ارومیه، ایران
۲- استاد، مرکز تحقیقات نفرولوژی و پیوند کلیه و گروه فیزیولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی ارومیه، ارومیه، ایران
۳- دانشیار، گروه فیزیولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی ارومیه، ارومیه، ایران
۴- استادیار، گروه فیزیولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی ارومیه، ارومیه، ایران
۵- استاد، گروه بیوشیمی، دانشکده پزشکی، دانشگاه علوم پزشکی ارومیه، ارومیه، ایران
نویسنده مسؤول: دکتر علیرضا شیرپور
Email: ashirpoor@yahoo.com