The Effects of Alcohol and Social Isolation on Yawning Behavior in Adult Male Rats, Treated with Opioid Receptor Agonist and Antagonist

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The number of 88 rats was chosen, after 21 days, they were ablated and categorized them as following groups:

The control group 1 has a cage of 8 rats in a social condition. After 6 weeks of saline injection (1mg / kg), the yawning behavior is recorded for 60 minutes. Group 2 in social conditions of receiving ethanol for 2 months, each serving of 600 mg / kg. Group 3 social isolation without treatment with agonist and antagonist, Opioid and alcohol drugs in a separate cage, each cage has one rat. After 6 weeks, It is recorded the yawning was cured for 60 minutes. Group 4 social isolation for 6 weeks and then treatment with alcohol agonist (1200 mg / kg) after 30 minutes of yawning behavior is recorded for 60 minutes. Group 5 Social isolation after 6 weeks of treatment with opioid drugs (5mg / kg of morphine) after 30 minutes of yawning behavior is recorded for 60 minutes. Group 6 social isolation after 6 weeks of morphine + alcohol antagonist treatment after 30 minutes of yawning behavior is recorded for 60 minutes. Group 7 social isolation followed by 6 weeks of treatment with antagonist alcohol (naltrexone), 10 mg / kg, after 30 minutes of yawning behavior is recorded for 60 minutes. Group 8 Social isolation after 6 weeks of treatment with antagonist morphine (naloxone) 1 mg / kg of bad breath after 30 minutes of yawning behavior is recorded for 60 minutes. Group 9 social isolation followed by 6 weeks of treatment with antagonist alcohol + morphine after 30 minutes for 60 minutes of yawning behavior is recorded for 60 minutes. Group 10 social isolation

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followed by 6 weeks of treatment with antagonist alcohol + morphine antagonist after 30 minutes for 60 minutes of yawning behavior is recorded for 60 minutes. Group 11 Social isolation after 6 treatments with antagonist morphine + alcohol followed after 30 minutes for 60 minutes of yawning behavior is recorded for 60 minutes. Injection of alcohol chronically reduces yawning, but alcohol injections in conditions of social isolation increase the number of yawns. Antagonist injection of naltrexone prior morphine in conditions of social isolation of yawning. Injection of morphine in conditions of social isolation does not change the number of yawning. Injection of morphine antagonist (naloxone) prior to antagonist of alcohol (naltrexone) in conditions of social isolation increases yawn. Also, the injection of morphine antagonist (naloxone) prior to alcohol into social isolation causes an increase in the number of yawns. Alcohol injection before morphine in conditions of social isolation increases the number of yawns. It seems that the stress of social isolation by decreasing alcohol and morphine function increases the number of yawns.

Keywords: Yawning; social isolation stress; opioid; alcohol.

1. INTRODUCTION

The astrocytic proteins GLAST, GLT-1 and GFAP are differentially regulated by social isolation and running. This fact illustrates the complex effect of physical activity and stress on the brain [1]. Paradoxically though, physical activity also activates the HPA axis functioning as a stressor [2,3] the astrocytes as main actors in the mechanisms underlying the stress and running response in the brain.

Impulsive action and impulsive choice were reduced in isolation-rearing IR rats under certain conditions, and a blunted response to d-amphetamine was found on these measures. These reductions in impulsivity contrast with locomotor hyperactivity normally shown in IR rats and the findings have implications for the utility of IR as a model of psychopathology [4].

In general, the research findings are in agreement with putative involvement of (brain-derived neurotrophic factor) BDNF in the pathogenesis of stress-related mental disorders. However, almost entirely absent are empirical data on the neurobiological mechanisms underlying the altered BDNF expression as well as the involvement of signaling pathways downstream of BDNF in chronically isolated animals [5].

In order to analyze the possible association between increased visceral fat amount and redox disequilibrium in isolated animals, we performed qPCR for specific genes encoding for antioxidant enzymes and ROS producers in visceral fat. We detected a decreased mRNA expression of Prdx1, known to be a crucial component of the antioxidant defense [6-7].

This study found that repeated (Maternal separation) MS induced enhanced locomotor activity in (an open-field test) the OFT and abated exploratory behavior of pubescent rats in both the LDB (the following order: light–dark box) and (an elevated plus-maze) EPM tests. (Early handling) EH abated the exploratory behavior of pubescent rats in (elevated plus-maze) the EPM test. These results indicated that MS significantly increased anxiety levels in individuals and that EH enhanced anxiety levels slightly, which was expressed by the abnormal behavior of fear memory. At the same time, female rats were more prone to showing anxiety-like behavior compared with male rats in the presence of high-intensity stimulation. Further research is needed to study the interaction between the duration and the frequency of separation at different ages [11,12,13].

Complete social isolation for a long period induces brain neuronal degenerations. It represents a risk factor especially when associated with AD; it increases DNA fragmentation and enhances the severity of AD development. Thus, socialization is advised especially with AD to avoid worsen or deterioration of the disease [14,15].

Neuropharmacological studies revealed that various neurotransmitters and neuropeptides are effective on yawning. The best known are adrenocorticotropin, α-melanocyte stimulating hormone (MSH) and related peptides, acetylcholine, dopamine, serotonin, excitatory
amino acids, oxytocin, gamma-amino butyric acid (GABA) and opioid peptides [16].

In rats and non-human primates yawning is androgen-dependent and sexually dimorphic, with more common in males than females [17].

Yawning occurs associated with increased electroencephalographic activity of the cortex, it has been also suggested that yawning is an ancestral vestige survived through evolution that occurs when attention is low and arousal needs to be increased [18].

Yawning is also contagious, as it can be also evoked in humans or non-human primates by a yawn produced by another subject of the same species. This led to suggest that yawning may be linked to empathy [19].

Locus coeruleus norepinephrine (LC-NE) dynamically regulates cued aversive learning and memory. We propose a model by which the LC-NE system can dynamically regulate cued Pavlovian fear conditioning and extinction based on the prevailing level of stress at the onset of learning. Under high levels of arousal the LC-NE system acts via α1 and β-ARs, a state that would favor elevated levels of fear and hinder new learning (for example, in the case of immediate extinction [20].

Yawning is one of the premonitory symptoms in migraine. Although the brainstem is highly linked to migraine biology, the real driver of attacks might be functional changes in hypothalamic-brainstem connectivity, explaining typical migraine premonitory symptoms such as fatigue and yawning, but also a typical association of attacks to circadian and menstrual cycles, all making the hypothalamus a possible regulating region of migraine attacks [21].

Consistent with a D3R mechanism, in the present studies, yawning produced by infusion of 1.0 g/kg ethanol was blocked by the D3R antagonist PG01037. Taken together, the data suggest that chronic ethanol exposure may increase D3R sensitivity, and this may play an indirect role in the ability of ethanol to produce yawning. Indeed, increased D3R binding in the hypothalamus has been reported using the D3R-preferential PET ligand PHNO, in alcohol-dependent patients [22].

The role of CLOCK and β-catenin in concert with GnIH neuronal activity may point toward a circadian component in the maintenance of regular neuronal activity under chronically stressful conditions, such as that induced by social isolation. Our findings continue to support the long-held consensus on the negative effects of social isolation and provide new insights into circadian regulation of GnIH neuronal activity [23].

Acute and chronic administration of morphine, an opioid receptor agonist, reduced almost completely penile erection, yawning and genital grooming induced by apomorphine. So there is interaction between dopamine and opiate receptors. Injecting morphine in chronic phase only enhances number of yawning. These results may be explained by the ability of opioid receptor agonists to markedly decrease oxytocin neurotransmission at central and posterior pituitary level. Dopamine in turn increases central oxytocinergic neurotransmission, leading to penile erection and yawning, as already suggested for apomorphine and other dopamine receptor agonists. Compounds enhancing yawning would probably increase the penile erection and genital grooming time behaviors in rat [24].

On the other hand, opiate interactions with both dopaminergic and cholinergic systems have been demonstrated [25].

2. MATERIALS AND METHODS

2.1 Drugs and Peptides

Morphine, Alcohol, Naloxone, Naltrexone, were purchased from Sigma (Germany). All other reagents were from available commercial sources.

2.2 Animals

Ninety 21-day-old male rats (250-300 g) were randomly allocated in groups of 3-4 under-controlled conditions of 23 ± 1°C temperature and 12 h light/dark cycle (lights on at 08:00), with ad libitum access to food and water. All experimental procedures were carried out between 09:00-13:00 h and were approved by the Animal Experimentation Ethics Committee of the University. Every effort was made to minimize animal suffering and the number of animals used.
2.3 Interventions

Morphine, naloxone, and naltrexone Wolk are dissolved in normal saline. All of the compounds were given intraperitoneal (IP) and 0.5 ml per animal. The control group receives the same amount of SCM.

2.4 Behavioral Studies

A sample of 88 Male rats (21-day post weaning) were chosen, and were put for 6 weeks in separate cages with black plastic buffers. Eight rats were put in one group of 8 rats in a single cage (n=8; the control group) and the rest were put in individual cages: one male rat in each cage. In group 1 or the control group (social conditions) 8 rats were put in one cage. They received saline carrier and their yawning behavior was recorded for 60 minutes. Group 2 (n=8; in social conditions) received ethanol (600 mg / kg) for 2 months on a daily basis; then their yawning behavior was recorded for 60 minutes. In Group 3 (n=8; in social isolation conditions), each rat was kept in a separate cage without treatment with agonist, antagonist, opioid, and alcohol drugs. After 6 weeks, their yawning behavior was recorded for 60 minutes. In Group 4(n=8; in separate cages) rats were kept in social isolation conditions for 6 weeks and then treated with alcohol agonist (1200 mg / kg). Thirty minutes after injection, their yawning behavior was recorded for 60 minutes. In Group 5, (n=8; in social isolation conditions) received alcohol (600 mg / kg) for 2 months on a daily basis; then their yawning behavior was recorded for 60 minutes. In Group 6, (n=8; in separate cages) rats were kept in social isolation for 6 weeks and then treated with alcohol agonist (naloxone) at a dose of 10 mg / kg. Thirty minutes after injection, their yawning behavior was recorded for 60 minutes.

In Group 7, (n=8; in separate cages) rats were kept in social isolation for 6 weeks followed by treatment with alcohol antagonist (naltrexone), at a dose of 10 mg / kg. Thirty minutes after injection, their yawning behavior was recorded for 60 minutes.

In Group 8, (n=8; in separate cages) rats were kept in social isolation for 6 weeks followed by treatment with morphine antagonist (naloxone) at a dose of 1 mg / kg. Thirty minutes after injection, their yawning behavior was recorded for 60 minutes.

In Group 9, (n=8; in separate cages) rats were kept in social isolation for 6 weeks followed by treatment with alcohol + morphine antagonist. Thirty minutes after injection, their yawning behavior was recorded for 60 minutes.

In Group 10, (n=8; in separate cages) rats were kept in social isolation for 6 weeks followed by treatment with alcohol antagonist + morphine antagonist. Thirty minutes after injection, their yawning behavior was recorded for 60 minutes.

In Group 11, (n=8; in separate cages) rats were kept in social isolation for 6 weeks followed by treatment with morphine + alcohol antagonist. Thirty minutes after injection, their yawning behavior was recorded for 60 minutes.

2.5 Statistical Analysis

The data were expressed as mean ± standard error of the mean. The parameters were evaluated by analysis of variance (ANOVA), followed by Tukey's test. The differences among the groups were assessed by Student’s t test, with a significance level of P<0.05.

3. Results

Average results and standard deviation of yawning Statistical analysis by one way ANOVA revealed a significant difference of P <0.001 between the experimental groups.

Compared to the Tukey Supplementary Medicines Taking Groups. The number of yawnings in the social isolation group was not significantly reduced compared to the control group and also in the chronic alcohol recipients group (n = 8) (P <0.001), there was a significant decrease compared to the control group and social isolation (n = 8) (0.01 / 0≥ P).

Also, there was a significant increase in the number of yawnings in naloxone - naloxone + naltrexone - naloxone + alcohol (n = 8) (P <0.05) compared to the control group and social isolation (sign # and * indicating significant Between the groups were at the level of (P ≥ 0.001) and (P ≥ 0.05).

There was no significant decrease in the number of yawning in the social isolation group compared to the control group in the Tuki supplementation group, and also in the chronic alcohol recipients
group (n = 8) (P <0.001), there was a significant decrease compared to the control group n = 8) 001/0 ≥ P). And in the naltrexone group, there was a significant increase in the number of yawns in comparison with control and alcohol, and also in the naltrexone + naloxone group, there was a significant increase in the number of yawning compared to the other groups (sign # and *) indicating significant Being between the groups was at the level of (P ≤ 0.001) and (P ≥ 0.05).

Compared to the Tukey Supplementary Medicines Taking Groups. The number of yawnings in the social isolation group was not significantly decreased compared to the control group and also in the chronic alcohol recipients group (n = 8) (P <0.001), there was a significant decrease in the control group n = 8 (P <0.001) In isolated isolate morphine receptor group, there was a significant increase in the number of yawnings compared to the chronic alcohol group and the isolated morphine + alcohol group showed a significant increase in the yawning threat compared to other groups (the # and * sign) showed significant Between the groups were at the level of (P ≥ 0.001) and (P ≥ 0.05).

Fig. 1. The number of yawning in the groups studied is shown

Fig. 2. Shows the number of yawns in the groups under review
Compared to the Tukey Supplementary Medicines Taking Groups. There was no significant decrease in the number of yawning in the social isolation group compared to the control group, and also in the chronic alcohol and alcoholic isolation group (P = 0.001), there was a significant decrease in the control group (n = 8) (P) and the Naltrexone group also showed a significant increase in the number of yawning compared to the control group. Also, in the naltrexone + alcohol group and the naloxone + naltrexone group, there was a significant increase in the number of yawning compared to the other groups (#) showed a significant difference between the groups at the level (P ≤ 0.001) and (P ≥ 0.05).

Compared to the Tukey Supplementary Medicines Taking Groups. The number of yawnings in the social isolation group was not significantly decreased compared to the control group and also in the chronic alcohol recipients group (n = 8) (P = 0.001), there was a significant decrease in the control group n = 8 (P = 0.001) and the morphine group also showed a significant increase in the number of yawnings compared to the control group and chronic alcohol. Also, the naloxone and naloxone + morphine - naloxone + alcohol - naloxone + Naltrexone also had a significant increase in the number of yawning compared to other groups (the mark # And *) showed significant difference between the groups studied at the level (P < 0.001) and (P < 0.05).

Compared to the Tukey Supplementary Medicines Taking Groups. The number of yawnings in the social isolation group was not significantly decreased compared to the control group and also in the chronic alcohol recipients group (n = 8) (P < 0.001), there was a significant decrease in the control group n = 8 (P < 0.001) and the naloxone group also showed a significant increase in the number of yawnings compared to the control group and chronic alcohol. Also, in the naltrexone + morphine group, naloxone + alcohol - Naltrexone + naloxone group also had a significant increase in the number of yawning compared to the other groups (sign # *) showed significant difference between the groups at the level (P ≤ 0.001) and (P ≥ 0.05).

Fig. 3. Shows the number of yawns in the groups examined
Fig. 4. Shows the number of yawns in the groups examined

Fig. 5. Shows the number of yawns in the groups under study

Fig. 6. Shows the number of yawns in the groups examined
4. DISCUSSION

In this study, the effects of alcohol and social isolation stress were investigated on yawning behavior in adult male rats treated with opioid receptor agonist and receptor antagonist. The results of this study indicate that chronic alcohol injections significantly reduced the number of yawns in the 60 minutes of delivery to the control group significantly. But alcohol injections in the context of social isolation have increased the number of yawning. Injection of morphine in conditions of social isolation does not change the number of yawning. Injection of naloxone before alcohol and injection of naltrexone before morphine in a social isolation enhances the number of yawning. In social isolation, the injection of alcohol prior to morphine indicates a significant increase in the number of yawning. Naloxone and naltrexone inoculation increased yawning in isolation conditions. However, naloxone pre-nerve injection and vice versa were not significantly different from naloxone and naltrexone injections alone in social isolation conditions. Several neurotransmitters involved in jaundice induction such as nitric oxide glutamate dopamine serotonin are GABA NMDA. By reducing glutamate and decreasing dopamine, alcohol can increase yawning in the brain [26]. Short-term exposure to alcohol inhibits NMDA and decreases yawning [27], as well as alcohol increases the function of inhibitors, such as GABA [28].

Increasing the GABA function, which is an inhibitor of neurotransmitter, reduces the number of yawns. Morphine decreases yawning by decreasing nitric oxide function [29,30]. Social isolation stress, on the other hand, decreases morphine and amphetamine function [31].

Alcohol Inhibits Excitatory Neurotransmission

Alcohol might induce sedative effects by reducing excitatory neurotransmission. The major excitatory neurotransmitters in the brain are the amino acids aspartate and glutamate, which act through both NMDA receptors—so named because they respond to the synthetic chemical N-methyl-D-aspartate—and non-NMDA receptors. Short-term exposure to intoxicating concentrations of alcohol appears to inhibit both NMDA and non-NMDA receptor activity, potentially resulting in sedation (Valenzuela and Harris 1997). As in the case of GABAA receptors, however, these excitatory receptors are relatively insensitive to intoxicating concentrations of alcohol under some experimental conditions, underscoring the need for more research in this area [26-27].

Although naltrexone has highest affinity for μ-opioid receptors, research has shown that naltrexone binds, in a dose-dependent manner first the μ-receptor, then the δ-receptor, and finally with the κ-opioid receptor. In fact, a PET study suggested that at a dose of 50 mg/day, naltrexone produces nearly complete (approximately 90%) blockade of μ-opioid receptor, compared to 20 to 35% blockade of δ-opioid receptors, and presumably lower occupancy of κ-opioid receptors. More recently, a
PET study of μ- and -opioid receptor blockade in naltrexone treated alcohol-dependent subjects by Weerts et al. [32].

The effects of hair transplant by opioid antagonists are reversed by naloxone. Administration of naloxone alone causes yawning, and the mechanism responsible for naloxone induced yawning is due to the removal of the orphan receptor opioid on the hairpiece. This effect of naloxone on yawning may be due to an inhibitory effect on opioid receptors in the hair (Turkmenis, 26). According to previous studies, social isolation stress, with increased oxidative stress, causes brain degeneration [20] and may be the cause of opiate-like effects such as Morphine or alcohol in the central nervous system.

5. CONCLUSION

The study found that social isolation stress prevented the strengthening of morphine and alcohol properties. While in social groups, reducing the number of yawning after the injection of alcohol, we observed a significant increase in yawning in isolated isolates of alcohol injection, or injection of alcohol and subsequent morphine. The social isolation stress of the effects of opioids and alcohol reverses the central nervous system, which requires further investigation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental procedures were approved by the Animal Experimentation Ethics Committee of the University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Novak CM, Burghardt PR, Levine JA. The use of a running wheel to measure activity in rodents: Relationship to energy balance, general activity, and reward. Neuroscience & Biobehavioral Reviews. 2012;36(3):1001–14.

2. Hackney AC. Stress and the neuroendocrine system: the role of exercise as a stressor and modifier of stress. Expert Rev Endocrinol Metab. 2006;1(6):783–92.

3. Fuss J, Ben Abdallah NMB, Vogt MA, Touma C, Pacifici PG, Palme R, et al. Voluntary exercise induces anxiety-like behavior in adult C57BL/6J rats correlating with hippocampal neurogenesis. Hippocampus. 2010;20(3):364–76.

4. Liu YP, Wilkinson LS, Robbins TW. ‘Waiting impulsivity’in isolation-reared and socially-reared rats: effects of amphetamine. Psychopharmacology. 2017;234(9-10):1587-601.

5. Murinová J, Hlaváčová N, Chmelová M, Riečanský I. The evidence for altered BDNF expression in the brain of rats reared or housed in social isolation: a systematic review. Frontiers in behavioral neuroscience. 2017;11:101.

6. Aeby E, Ahmed W, Redon S, Simanis V, Lingner J. Peroxiredoxin 1 protects telomeres from oxidative damage and preserves telomeric DNA for extension by telomerase. Cell Reports. 2016;17(12):3107-14.

7. Behtash N, Kargarzadeh F, Shafaroudi H. Analgesic effects of seed extract from Petroselinum crispum (Tagetes minuta) in animal models. Toxicology Letters. 2008;180:S127-8.

8. Moein M, Moein S, Farmani F, Rozbehian S, Sabahi Z. Examination the antioxidant potentials and antidiabetic properties of phenolic extracts of some Iranian honeys. Journal of Nephropharmacology. 2021;11(1):e6-e6.

9. Shie Morteza, Hayati, Z, Namazi, N, Abdallahimajd, F. Efficacy and safety of oral silymarin in comparison with oral doxycycline and their combination therapy in the treatment of acne vulgaris. Dermatologic Therapy. 2019;32:e13095. Available:https://doi.org/10.1111/dth.13095

10. Behtash N, Shafaroudi H, Khorsangani ZN. Analgesic effect of Calendula officinalis flowers extract in mice. Toxicology Letters. 2010(196):S251.

11. Ieraci A, Mallei A, Popoli M. Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. Neural Plasticity; 2016.
12. Babamiri S, Mojani Qomi MS, Shiehmorteza M. The efficacy of Ganoderma lucidum in overweight individuals: A randomized placebo-controlled trial. Mediterranean Journal of Nutrition and Metabolism. 2022;15(2):263-271.
13. Nikraftar NS, Feyzi YF, Ramzani F, Nikbakht-Zadeh M, Amini M, Arezoomandan M, Shiehmorteza M, Arezoomandan R. Comparison of psychological symptoms and cognitive functions in patients under maintenance treatment with methadone or buprenorphine, current opioid users and healthy subjects. Asian Journal of Psychiatry. 2021;58:102603.
14. Ali AA, Khalil MG, Abu-Elfotuh K. Study on social isolation as a risk factor in development of Alzheimer's disease in rats. Brain Disord Ther. 2017;6(230):2.
15. Behtash N, Nazarikhorasgani Z, Jalali T, Latifi SM, Goudarzi M. Serum vitamin E levels and risk for coronary arteries atherosclerosis, Ahvaz, Iran. Toxicology Letters. 2012;211:S212.
16. Patatanian E, Williams NT. Drug-induced yawning—a review. Annals of Pharmacotherapy. 2011;45(10):1297-301.
17. Graves FC, Wallen K. Androgen-induced yawning in rhesus monkey females is reversed with a nonsteroidal anti-androgen. Hormones and Behavior. 2006;49(2):233-6.
18. Guggisberg AG, Mathis J, Herrmann US, Hess CW. The functional relationship between yawning and vigilance. Behavioural Brain Research. 2007;179(1):159-66.
19. Anderson JR, Myowa-Yamakoshi M, Matsuzawa T. Contagious yawning in chimpanzees. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2004;271(Suppl_6):S468-70.
20. Giustino TF, Maren S. Noradrenergic modulation of fear conditioning and extinction. Frontiers in behavioral neuroscience. 2018;12:43.
21. Krestel H, Bassetti CL, Walusinski O. Yawning—its anatomy, chemistry, role, and pathological considerations. Progress in Neurobiology. 2018;161:61-78.
32. Ray LA, Chin PF, Miotto K. Naltrexone for the treatment of alcoholism: clinical findings, mechanisms of action, and pharmacogenetics. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders). 2010;9(1):13-22.

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