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Research report

Conessine, an H_3 receptor antagonist, alters behavioral and neurochemical effects of ethanol in mice

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HIGHLIGHTS

- Conessine, an H_3 receptor antagonist, exacerbated ethanol-induced psychostimulation.
- Pretreatment with conessine did not alter ethanol CPP.
- Conessine had reinforcing proprieties *per se*.
- Norepinephrine and serotonin might be related to the reinforcing proprieties.
- Dopamine might be related to the conessine exacerbation of ethanol psychostimulation.

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ABSTRACT

Ethanol abuse potential is mainly due to its reinforcing properties, crucial in the transition from the recreational to pathological use. These properties are mediated by mesocorticolimbic and nigrostriatal dopaminergic pathways and neuroadaptations in these pathways seem to be responsible for addiction. Both pathways are modulated by other neurotransmitters systems, including neuronal histaminergic system. Among the histamine receptors, H_3 receptor stands out due to its role in modulation of histamine and other neurotransmitters release. Thus, histaminergic system, through H_3 receptors, may have an important role in ethanol addiction development. Aiming to understand these interactions, conessine, an H_3 receptor antagonist, was given to mice subjected to the evaluation of ethanol-induced psychostimulation, ethanol CPP and quantification of norepinephrine, dopamine, serotonin and their metabolites in mesocorticolimbic and nigrostriatal pathways following acute ethanol treatment. Systemic conessine administration exacerbated ethanol effects on locomotor activity. Despite of conessine reinforcing effect on CPP, this drug did not alter acquisition of ethanol CPP. Ethanol treatment affects the serotoninergic neurotransmission in the ventral tegmental area, the dopaminergic neurotransmission in the pre-frontal cortex (PFC) and caudate-putamen nucleus (CPu) and the noradrenergic neurotransmission in the CPu. In the PFC, conessine blocked ethanol effects on dopaminergic and noradrenergic neurotransmission. The blockade of H_3 receptors and ethanol seem to interact in the modulation of dopaminergic neurotransmission of nigrostriatal pathway, decreasing dopamine metabolites in substantia nigra. In conclusion, conessine was able to change psychostimulant effect of ethanol, without altering its reinforcing properties. This exacerbation of ethanol-induced psychostimulation would be related to alterations in dopaminergic neurotransmission in the nigrostriatal pathway.

1. Introduction

Every year the abuse of alcoholic beverages is responsible for about 3.3 million deaths [1]. Several injuries are causally linked to alcohol use and ethanol addiction is the major risk factor for the most of them [2]. The abuse potential of psychoactive substances, including ethanol, is mainly due to their reinforcing proprieties, a characteristic responsible for the initial search and repeated

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consumption of these substances that can lead to alcohol-related injuries and development of addiction in susceptible subjects.

The most important neural pathway related to the reinforcing proprieties, whether for natural stimuli or drugs of abuse is the dopaminergic mesocorticolimbic pathway. It consists of dopaminergic neurons located in ventral tegmental area (VTA) that project to the nucleus accumbens (NAc) and pre-frontal cortex (PFC) [3]. Other dopaminergic pathway important in the processes related to addiction is the nigrostriatal pathway, which is comprises neurons from the substantia nigra (SN) pars compacta to the caudate-putamen nucleus (CPu). This last neuronal pathway and theirs connections have an important role in habit formation, crucial feature of addiction development [4,5].

Among the aminergic systems that exist in mammalian brain, the histaminergic system acts modulating sensory information according to individual's memories and physiology. It consists of a small neuronal group that projects to the entire nervous system. The histamine containing neurons are restricted to the posterior part of the hypothalamus, the tuberomammilary nucleus, which sends projections to both mesocorticolimbic and nigrostriatal pathways. Four receptors are known for histamine, named H_{1-4}. They are G-protein coupled receptors highly distributed in central nervous system [6]. The H_3 receptor acts mainly as an inhibitory autorreceptor in the central nervous system through $G_{i/0}$ protein. It controls the release of many neurotransmitters in addition to histamine, such as dopamine, norepinephrine, serotonin, GABA and acetylcholine. This receptor is highly expressed in addiction-related brain areas, such as NAc and PFC [6].

There are many H_3 receptor antagonists available for research use. Among them, conessine is one of the most selective for H_3 receptors, with high affinity. It's derived from the bark and seeds of plants from Apocynaceae family, especially *Holarrhena antidysenterica*, freely crosses blood-brain barrier and has slow clearence in central nervous system [7,8]. Different from the most common compounds, conessine is a non-imidazole H_3 antagonist, probably having no action in enzymes of cytochrome P450 [9]. This is a desirable characteristic of this drug for the study of mechanisms involved in ethanol addiction, since it probably does not affect alcohol metabolism.

Post-mortem studies using the brain of alcoholics suggest a role of the histaminergic system on ethanol addiction. The brain of such individuals has increased histamine metabolites [10]. Furthermore, some polymorphisms were found in genes that codify enzymes of histamine synthesis and metabolism in ethanol addicted [11,12]. In animal models of substance abuse, drugs which act modulating histaminergic system both aggravate and ameliorate some parameters of addiction. For example, systemic administration of a histamine precursor aggravate lorazepam withdrawal syndrome while H_1 receptor antagonists attenuate it. In this same study, the H_3 antagonist thioperamide administration increased lorazepam withdrawal syndrome [13]. Histamine levels are higher in a rat lineage selected for high ethanol consumption and preference. When these animals are treated with the H_3 receptor antagonist thioperamide, their consumption decrease significantly [14]. Considering these evidences about the role of histaminergic system in addiction and the importance of H_3 receptors in the control of neurotransmitters release, we evaluated the effects of conessine treatment in the psychostimulant and reinforcing effects of ethanol, and the related neurochemical alterations in mice.

2. Material and methods

2.1. Subjects

One hundred fifty seven male Swiss mice (Center of Bioterism and Animal Experimentation, Federal University of Uberlândia, Uberlândia, MG-BRA; 30–35 g) were transferred to our animal facility at least seven days before the start of the experiments and were housed within groups of four or five per cage. The room was maintained at a temperature of $23 \pm 2\,^{\circ}C$ on a 12:12 h light/dark cycle with *ad libitum* water and food access. All experiments were performed during the light phase of the cycle and animals were randomly tested across this time period. The experimental protocol was approved by the Ethical Committee for the Animal Utilization of Federal University of Uberlândia (CEUA 029/13) and the experiments were conducted according to the principles of the National Council for Animal Experiments Control (CONCEA), based on NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs

Conessine (Sigma Aldrich, St. Louis, MO-USA) was diluted in extra virgin olive oil (Sandéleh Alimentos, Sorocaba, SP-BRA) as vehicle and administered subcutaneously (0.1 mL/10 g) at the doses of 0.1, 1.0 or 10.0 mg/kg. Ethanol was diluted in saline (NaCl 0.9%) and administered intraperitoneally at the dose of 2.0 g/kg in the evaluation of the locomotor activity and neurotransmitters quantification experiments and at the dose of 1.0 g/kg in conditioned place preference (CPP) experiment. The drug injected dose was obtained from 20% (v/v) ethanol solution in the experiments for the evaluation of locomotor activity and from 10% (v/v) ethanol solution in the CPP experiment.

2.3. Evaluation of locomotor activity

Locomotor activity was evaluated by the measurement of the distance travelled (in meters) by mice after treatments in the open field (OF) apparatus. The OF is a circular arena with black floor (Master One, Ribeirão Preto-SP, Brazil), 30 cm in diameter, surrounded by 30 cm high transparent walls.

In the day of the experiment, the animals received conessine (0.1, 1.0 or 10.0 mg/kg) or vehicle and one hour later ethanol (2.0 g/kg) or saline. The animals (N = 8–9 animals per group) were then immediately placed in the OF and their locomotor activity measured during 30 min by the behavioral analysis software ANY-maze (Stoelting Co., Wood Dale, IL-USA). Animal's movements were captured by a HD camera fixed above four OF apparatuses and connected to a computed with the behavioral analysis software. Distance travelled was analyzed as total distance covered during the 30 min and also in blocks of 5 min for a temporal analysis of ethanol and conessine effects.

2.4. Conditioned place preference

CPP was evaluated as described by Ref. [15] with small modifications. During the even days of conditioning phase (days 2, 4, 6 and 8), animals were s.c. treated with conessine (1.0 or 10.0 mg/kg) or vehicle one hour before i.p. ethanol (1.0 mg/kg) or saline administration, after which they were immediately put in the CPP apparatus. On odd days of conditioning phase (days 3, 5, 7, 9) animals received only vehicle one hour before saline administration, after which they were immediately put in the CPP apparatus. Control animals received vehicle and saline every day. Preconditioning and postconditioning tests were conducted as already described by [15]. Time spent (in seconds) in each compartment during preconditioning and postconditioning tests was measured by the behavioral analysis software ANY-maze (Stoelting Co., Wood Dale, IL-USA) and the compartment preference in postconditioning test was compared to the initial preference in preconditioning test (N = 9–10 animals per group).

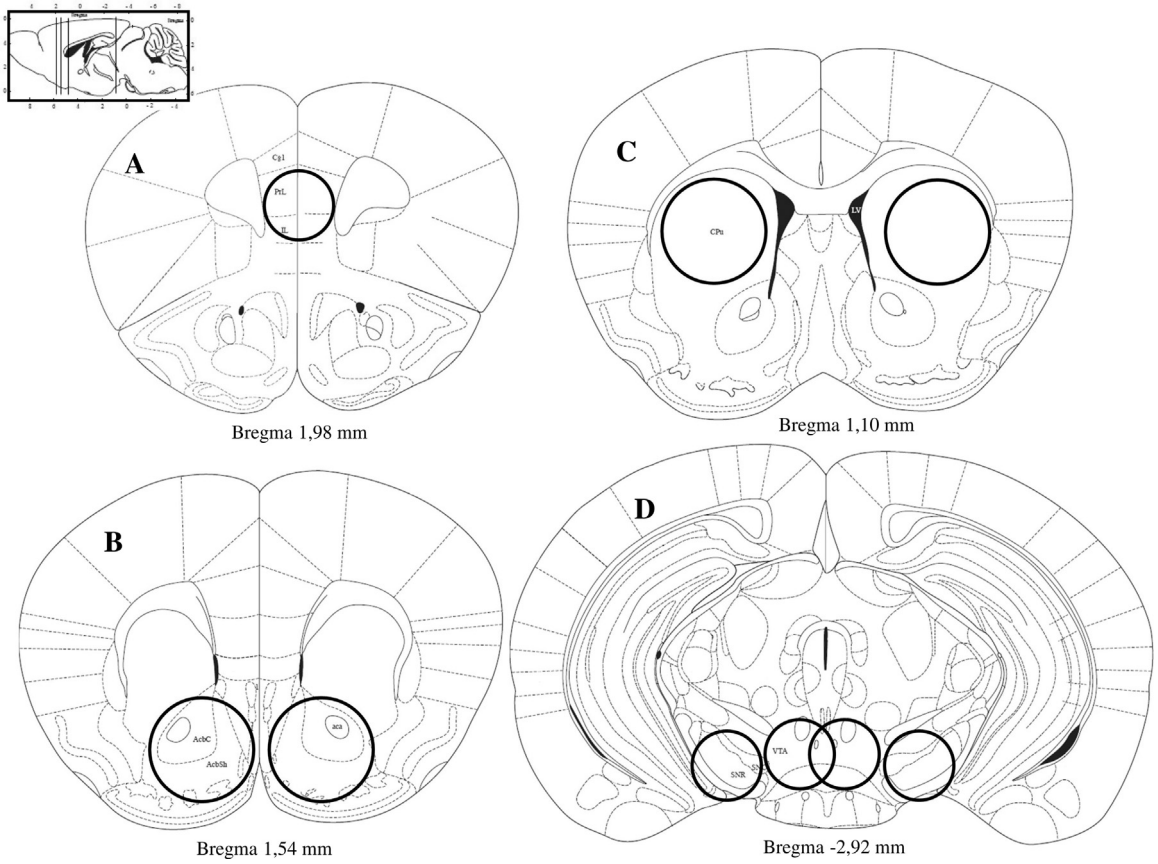

Fig. 1. Brain regions extracted for the quantification of neurotransmitters. Circles represents dissection sites. (A)Pre-frontal cortex (Bregma 1.98 mm) (B)Nucleus Accumbens (Bregma 1.54 mm), (C)Caudate-putamen nucleus (Bregma 1.10 mm), (D) Ventral tegmental area and substantia nigra (Bregma−2.92 mm). Cg1, cingulate cortex; Prl, prelimbic cortex; IL, infralimbic cortex; AcbC, core part of nucleus accumbens; AcbSh, shell part of nucleus accumbens; aca, anterior commissure; CPu, caudate-putamen nucleus; LV, lateral ventricle; SNR; substantia nigra, pars reticulata; VTA, ventral tegmental area.
Adapted from Paxinos and Franklin atlas of stereotaxic coordinates (2001).

2.5. Neurotransmitters quantification by HPCL

Following decapitation, the brains were rapidly removed from the skull and frozen in liquid nitrogen (−196 °C) and then kept at −80 °C until dissection. In a cryostat at −20 °C, brains were coronally sectioned to find target areas according to stereotaxic coordinates from the Atlas of Paxinos and Franklin [16] as follows: PFC, Bregma 1.98 mm; NAc, Bregma 1.54 mm, CPu, Bregma 1.10 mm; VTA and SN, Bregma −2.92 mm. Samples of 1 mm thickness were then removed with a flat-tipped needle with 1.0 mm of diameter for PFC, VTA and SN samples and 1.4 mm of diameter for NAc and CPu samples (Fig. 1).

The technique for quantification of norepinephrine, dopamine, serotonin, homovanillic acid (HVA), 3,4 dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5HIAA) was based on assays described by Ref. [15] with modifications. The volumes of perchloric acid were as follows: 60 μL for PFC, VTA and SN and 80 μL for NAc and CPu. The limit of detection and quantification, as already standardized in this instrument was respectively, for norepinephrine: 3.04 and 4.61, for dopamine 0.77 and 1.66, for DOPAC: 1.09 and 1.62, for HVA: 3.35 and 5.04, for serotonin: 1.67 and 2.48 and for 5HIAA: 1.85 and 2.76 ng/mL. The concentrations of the substances were corrected according to the mass of tissue from the dissected samples and were expressed as ng of substance per milligram of tissue. Based on the concentration of these substances, dopamine turnover was calculated as the ratio (DOPAC + HVA)/dopamine and serotonin turnover as 5-HIAA/serotonin. Total dopamine metabolites were calculated as the sum of DOPAC + HVA (N = 4–10 animals per group).

2.6. Statistical analysis

Statistical analyses were performed using Statistica software (StatSoft Inc., Tulsa, OK-USA). The results of the locomotor activity were analyzed by three-way ANOVA considering the factors treatment (conessine *vs* vehicle), ethanol (ethanol *vs* saline) and time (6 intervals of 5 min) factors. The results of CPP were analyzed by three-way ANOVA considering the factors treatment (conessine *vs* vehicle), ethanol (ethanol *vs* saline) and conditioning (preconditioning *vs* postconditioning) factors. The results of neurotransmitters quantification were analyzed by two-way ANOVA considering treatment (conessine *vs* vehicle) and ethanol (ethanol *vs* saline) factors. In cases witch ANOVA showed significant differences ($p \leq 0.05$) Duncan *post hoc* test was performed.

3. Results

3.1. Experiment 1: effects of conessine and ethanol treatment on locomotor activity

The three-way ANOVA revealed significant effect of the factor treatment ($F_{3,58} = 2.81$, $p < 0.05$), ethanol ($F_{1,58} = 36.05$, $p < 0.001$), time ($F_{5,290} = 112.71$, $p < 0.001$) and the interaction between factors ethanol and time ($F_{5,290} = 25.70$, $p < 0.001$). The interaction between factors treatment and ethanol showed a tendency to be significant ($F_{3,58} = 2.30$, $p = 0.08$). During the first 5 min of test, the Duncan *post hoc* test revealed an increase in distance travelled in all groups that received ethanol when compared to the groups that received saline

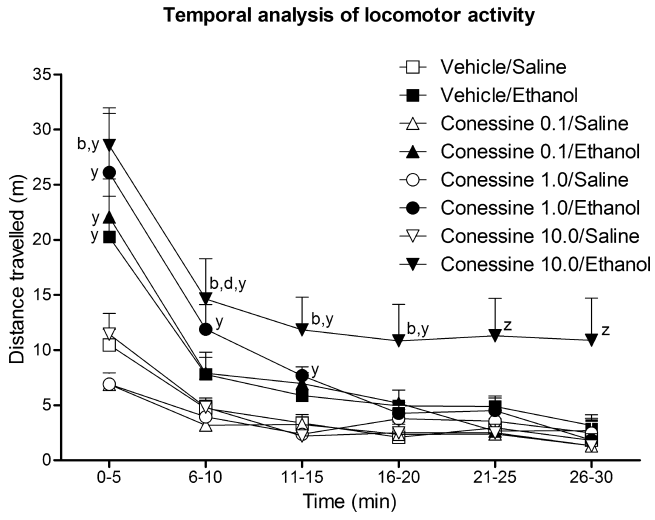

Fig. 2. Effects of conessine treatment on ethanol-induced psychostimulation in mice. Points represent means + SE of distance travelled in apparatus. N = 8–9 animals per group. Each animal received conessine s.c. at correspondent dose or vehicle and one hour after received ethanol 2 g/kg i.p. or saline, after which was immediately placed in the apparatus for 30 min. b, $p \leq 0.05$ relative to Vehicle/Ethanol group. d, $p \leq 0.05$ relative to Conessine 0.1/Ethanol group. y, $p \leq 0.05$ relative to experimental groups that received saline. z, $p \leq 0.05$ relative to all the other experimental groups.

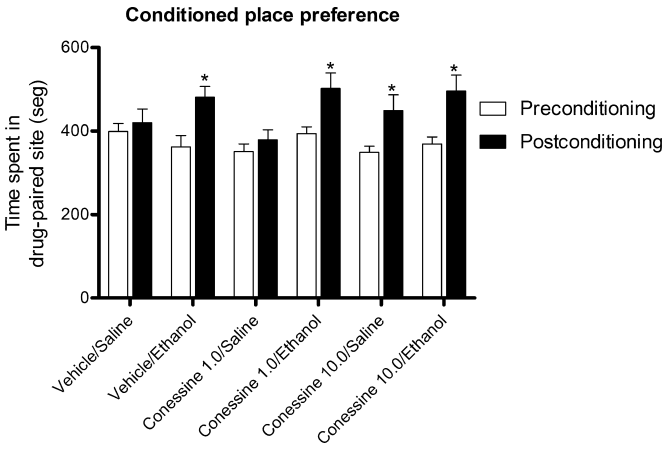

Fig. 3. Effects of conessine and ethanol treatment on conditioned place preference in mice. Bars represent means + SE of time spent in ethanol-paired site. N = 9–10 animals per group. Each animal received conessine s.c. at correspondent dose or vehicle during even days of conditioning phase (8 days long) and one hour after received ethanol 1 g/kg, i.p. or saline, after which was immediately confined in the nonpreferred compartment of the apparatus for 20 min. During odd days, the animals of all groups received vehicle and saline before being confined in the preferred compartment of the apparatus. *$p \leq 0.05$ when the difference between preconditioning and postconditioning was significant.

($p \leq 0.01$). Furthermore, the distance travelled by the animals of the group Conessine 10.0 mg/kg/Ethanol were significant greater than the locomotion of the animals of Vehicle/Ethanol group ($p < 0.01$) and Conessine 0.1 mg/kg/Ethanol group ($p < 0.05$). In the 6–10 min interval, the Duncan *post hoc* test showed that the distance travelled by the group Conessine 10.0 mg/kg/Ethanol was statistically greater than the distance travelled by all the other groups ($p < 0.05$) except by the group Conessine 1.0 mg/kg/Ethanol ($p = 0.30$). The distance travelled by the group Conessine 1.0 mg/kg/Ethanol was only statistically greater than saline challenged groups ($p < 0.05$). In 11–15 and 16–20 min interval, the Duncan *post hoc* test revealed that the group Conessine 10.0 mg/kg/Ethanol showed statistically greater locomotor activity in comparison to all the other experimental groups ($p < 0.05$), except from Conessine 0.1 mg/kg/Ethanol and Conessine 1.0 mg/kg/Ethanol groups. From the minute 21 ahead (21–25; 26–30 intervals) only the group Conessine 10.0 mg/kg/Ethanol showed an increase in locomotor activity, that was significant greater than locomotor activity of all the other groups ($p \leq 0.05$) (Fig. 2).

3.2. Experiment 2: effects of conessine and ethanol treatment on conditioned place preference

In the experiment of CPP two doses of conessine was tested: 1.0 mg/kg and 10.0 mg/kg. Fig. 3 shows the results obtained. The three-way ANOVA revealed significant effect of the factor ethanol ($F_{1,52} = 13.47$, $p < 0.001$), conditioning ($F_{1,52} = 37.65$, $p < 0.001$) and for the interaction between these two factors ($F_{1,52} = 9.52$, $p < 0.01$). The Duncan *post hoc* test showed ethanol conditioning (significant increase in the time spent in drug-paired site between preconditioning and postconditioning phases) for the three ethanol treated groups (Vehicle/Ethanol, Conessine 1.0 mg/kg/Ethanol and Conessine 10.0 mg/kg/Ethanol, $p < 0.01$) with no difference among then ($p > 0.05$). Interestingly, there was conditioning to conessine 10 mg/kg *per se* (pre *vs* postconditioning of group Conessine 10 mg/kg/Saline, $p < 0.05$).

3.3. Experiment 3: neurochemical evaluation of mesocorticolimbic and nigrostriatal pathways after conessine and ethanol treatment

3.3.1. Dopamine and dopamine-related alterations (Table 1)

Two-way ANOVA did not show significant effects of any factors for dopamine, DOPAC, HVA or DOPAC + HVA concentrations in the PFC ($p > 0.05$). However for dopaminergic turnover, the interaction between the factors treatment and ethanol showed a tendency of significance ($F_{1,24} = 3.01$, $p = 0.09$). Duncan *post hoc* test revealed that the group Vehicle/Ethanol showed greater dopaminergic turnover than the group Vehicle/Saline.

Although no alterations in dopamine, DOPAC and HVA concentrations and dopaminergic turnover were found in CPu of the animals ($p > 0.05$), the sum of the DOPAC + HVA concentration showed a tendency of decrease in ethanol groups. The two-way ANOVA revealed a tendency of significant effect of ethanol factor ($F_{1,22} = 3.49$, $p = 0.07$).

HVA content and the sum of DOPAC + HVA concentrations in the SN of the animals were altered by the treatment. Two-way ANOVA of HVA concentration showed significant effect of the treatment factor ($F_{1,28} = 14.74$, $p < 0.001$), ethanol factor ($F_{1,28} = 9.89$, $p < 0.01$) and the interaction between these two factors ($F_{1,28} = 4.92$, $p < 0.05$). The Duncan *post hoc* test showed that the HVA concentration of the Conessine/Ethanol group was lower than all the other groups ($p < 0.05$). Similar results were found for DOPAC + HVA concentration. Two-way ANOVA showed significant effect of the treatment factor ($F_{1,29} = 11.03$, $p < 0.01$), ethanol factor ($F_{1,29} = 5.38$, $p < 0.05$) and the interaction between these two factors ($F_{1,29} = 6.83$, $p < 0.01$). The Duncan *post hoc* test showed that the DOPAC + HVA concentration of the Conessine/Ethanol group was lower than all the other groups ($p < 0.05$). No significant alterations were found for dopamine and DOPAC concentrations and dopaminergic turnover in the SN of the animals ($p > 0.05$).

There were no significant alterations on dopamine, DOPAC, HVA, DOPAC+HVA content and dopamine turnover in the NAc and VTA of the animals.

3.3.2. Norepinephrine, serotonin and 5HIAA alterations (Table 2)

Norepinephrine content in PFC was altered by conessine or ethanol injection. The two-way ANOVA showed significant effect

Table 1
Effects of conessine (10 mg/kg) and ethanol (2 g/kg) administration on DA, DOPAC and HVA concentration (ng/mg of tissue) and dopamine turnover in mice.

	PFC				
	DA	DOPAC	HVA	DOPAC +HVA	DA turnover
Vehicle/Saline	0.31 ± 0.063	0.11 ± 0.029	0.09 ± 0.012	0.20 ± 0.040	0.61 ± 0.096
Conessine/Saline	0.23 ± 0.042	0.12 ± 0.017	0.09 ± 0.006	0.21 ± 0.021	0.86 ± 0.163 *
Vehicle/Ethanol	0.26 ± 0.057	0.09 ± 0.015	0.08 ± 0.005	0.17 ± 0.019	1.09 ± 0.170
Conessine/Ethanol	0.25 ± 0.033	0.11 ± 0.017	0.09 ± 0.008	0.19 ± 0.024	0.84 ± 0.117
	NAc				
	DA	DOPAC	HVA	DOPAC +HVA	DA turnover
Vehicle/Saline	0.88 ± 0.213	0.58 ± 0.058	0.31 ± 0.024	0.85 ± 0.073	1.18 ± 0.259
Conessine/Saline	0.66 ± 0.099	0.52 ± 0.042	0.28 ± 0.016	0.77 ± 0.046	1.57 ± 0.384
Vehicle/Ethanol	0.70 ± 0.153	0.56 ± 0.060	0.30 ± 0.031	0.87 ± 0.090	1.34 ± 0.237
Conessine/Ethanol	0.80 ± 0.136	0.55 ± 0.057	0.32 ± 0.033	0.86 ± 0.088	1.12 ± 0.207
	VTA				
	DA	DOPAC	HVA	DOPAC +HVA	DA turnover
Vehicle/Saline	0.01 ± 0.005	0.05 ± 0.003	0.06 ± 0.007	0.10 ± 0.009	10.41 ± 2.536
Conessine/Saline	0.02 ± 0.009	0.04 ± 0.005	0.06 ± 0.006	0.11 ± 0.008	12.30 ± 4.632
Vehicle/Ethanol	0.02 ± 0.004	0.04 ± 0.004	0.05 ± 0.006	0.10 ± 0.009	5.13 ± 1.131
Conessine/Ethanol	0.02 ± 0.005	0.03 ± 0.006	0.06 ± 0.005	0.09 ± 0.006	14.13 ± 7.535
	CPu				
	DA	DOPAC	HVA	DOPAC +HVA	DA turnover
Vehicle/Saline	1.38 ± 0.390	0.34 ± 0.035	0.22 ± 0.015	0.56 ± 0.041	0.62 ± 0.200
Conessine/Saline	1.03 ± 0.184	0.38 ± 0.048	0.21 ± 0.012	0.56 ± 0.036 *	0.57 ± 0.085
Vehicle/Ethanol	1.31 ± 0.304	0.33 ± 0.037	0.21 ± 0.019	0.54 ± 0.050	0.52 ± 0.112
Conessine/Ethanol	1.23 ± 0.103	0.26 ± 0.010	0.18 ± 0.007	0.44 ± 0.014	0.35 ± 0.023
	SN				
	DA	DOPAC	HVA	DOPAC +HVA	DA turnover
Vehicle/Saline	0.03 ± 0.010	0.05 ± 0.003	0.05 ± 0.003	0.10 ± 0.003	14.92 ± 8.699
Conessine/Saline	0.03 ± 0.008	0.05 ± 0.006	0.05 ± 0.004	0.09 ± 0.009 *	10.50 ± 7.191
Vehicle/Ethanol	0.03 ± 0.009	0.05 ± 0.003	0.05 ± 0.002 *	0.10 ± 0.004	14.23 ± 8.776
Conessine/Ethanol	0.01 ± 0.002	0.04 ± 0.005	0.03 ± 0.002	0.06 ± 0.005	22.48 ± 12.134

Data are expressed as Mean ± SE (N = 4–10 per group). DA Turnover = (DOPAC + HVA)/DA.
PFC (Prefrontal Cortex), NAc (Nucleus Accumbens), VTA (Ventral Tegmental Area), CPu (Caudate Putamen), SN (Substantia Nigra).
*$p \leq 0.05$.

of the interaction between the factors ethanol and treatment ($F_{1,23} = 4.61$, $p < 0.05$). Duncan *post hoc* test showed that the concentration of norepinephrine in PFC of the groups Vehicle/Ethanol and Conessine/Saline was smaller than the concentration of the group Vehicle/Saline.

The two-way ANOVA showed significant effect of ethanol factor for serotonin concentration ($F_{1,26} = 5.21$, $p < 0.05$) and for serotoninergic turnover ($F_{1,19} = 8.08$, $p < 0.01$) in VTA of the animals. Serotonin concentration in ethanol groups showed an increase relative to saline groups while serotonin turnover showed a decrease in ethanol groups compared to saline groups. There are no significant alterations in 5HIAA content in this region ($p > 0.05$).

Norepinephrine concentration in CPu showed an increase in groups treated with ethanol. The two-way ANOVA showed significant effect for ethanol factor ($F_{1,26} = 9.46$, $p < 0.01$) with no interaction.

There were no significant alterations on serotonin and 5HIAA content and serotonin turnover in the PFC, NAc, CPu and SN of the animals. With regard to norepinephrine content, there were no significant effects in NAc, VTA and SN.

4. Discussion

Our results suggest an interaction between the acute effects of ethanol administration and neuronal histaminergic system in mice. Systemic treatment with the H_3 receptor antagonist conessine increased ethanol-induced psychostimulation in a dose-dependent manner, mainly prolonging ethanol effects on locomotion. On the other hand, reinforcing proprieties of ethanol were not altered by conessine despite the conditioning effects of conessine at the dose of 10 mg/kg *per se*. The interaction between the blockade of H_3 receptors on ethanol effects seem to involve the modulation of dopaminergic neurotransmission of nigrostriatal pathway, decreasing dopamine metabolites in SN. Ethanol treatment affected the serotoninergic neurotransmission in the VTA, the dopaminergic neurotransmission in the PFC and CPu and the noradrenergic content in the CPu. In the PFC, conessine blocked ethanol effects on dopaminergic and noradrenergic neurotransmission.

In the literature there are conflicting results regarding the effects of H_3 receptor antagonists on ethanol-induced psychostimulation. There are results showing both an increase [17] or decrease [18–20] of ethanol-induced psychostimulation. There are also results

Table 2
Effects of conessine (10 mg/kg) and ethanol (2 g/kg) administration on 5HT, 5HIAA and NE concentration (ng/mg of tissue) and serotonin turnover in mice.

	5HT	5HIAA	5HT turnover	NE
CPF				
Vehicle/Saline	0.07 ± 0.017	0.17 ± 0.017	3.94 ± 1.302	0.07 ± 0.009
Conessine/Saline	0.03 ± 0.010	0.15 ± 0.017	4.15 ± 1.064	0.05 ± 0.006 *
Vehicle/Ethanol	0.06 ± 0.023	0.15 ± 0.011	3.02 ± 1.074	0.05 ± 0.006
Conessine/Ethanol	0.06 ± 0.019	0.15 ± 0.017	5.60 ± 2.023	0.06 ± 0.003
NAc				
	5HT	5HIAA	5HT turnover	NE
Vehicle/Saline	0.07 ± 0.025	0.40 ± 0.016	8.63 ± 3.024	0.13 ± 0.012
Conessine/Saline	0.06 ± 0.017	0.38 ± 0.028	10.49 ± 4.240	0.11 ± 0.011
Vehicle/Ethanol	0.03 ± 0.016	0.37 ± 0.018	9.84 ± 2.303	0.11 ± 0.018
Conessine/Ethanol	0.05 ± 0.024	0.37 ± 0.015	6.01 ± 2.030	0.12 ± 0.012
ATV				
	5HT	5HIAA	5HT turnover	NE
Vehicle/Saline	0.07 ± 0.027	0.48 ± 0.022	11.00 ± 3.859	0.05 ± 0.010
Conessine/Saline	0.07 ± 0.021	0.46 ± 0.073	5.85 ± 1.386 *	0.04 ± 0.009
Vehicle/Ethanol	0.15 ± 0.051	0.45 ± 0.052	3.18 ± 0.783	0.04 ± 0.009
Conessine/Ethanol	0.15 ± 0.037	0.50 ± 0.030	3.02 ± 0.468	0.05 ± 0.011
CPu				
	5HT	5HIAA	5HT turnover	NE
Vehicle/Saline	0.11 ± 0.022	0.28 ± 0.019	2.55 ± 0.357	0.06 ± 0.008
Conessine/Saline	0.10 ± 0.015	0.27 ± 0.011	2.81 ± 0.394	0.08 ± 0.008 *
Vehicle/Ethanol	0.10 ± 0.018	0.27 ± 0.016	2.85 ± 0.436	0.09 ± 0.007
Conessine/Ethanol	0.11 ± 0.008	0.25 ± 0.011	2.49 ± 0.212	0.09 ± 0.005
SN				
	5HT	5HIAA	5HT turnover	NE
Vehicle/Saline	0.30 ± 0.124	0.59 ± 0.043	6.67 ± 2.525	0.05 ± 0.008
Conessine/Saline	0.22 ± 0.070	0.63 ± 0.047	2.76 ± 0.483	0.05 ± 0.010
Vehicle/Ethanol	0.21 ± 0.076	0.54 ± 0.019	8.52 ± 3.432	0.07 ± 0.008
Conessine/Ethanol	0.14 ± 0.039	0.54 ± 0.030	5.60 ± 1.881	0.06 ± 0.005

Data are expressed as Mean ± SE (N = 4–10 per group). 5HT Turnover = 5HIAA/5HT.
PFC (Prefrontal Cortex), NAc (Nucleus Accumbens), VTA (Ventral Tegmental Area), CPu (Caudate Putamen), SN (Substantia Nigra).
*$p \leq 0.05$.

showing no effects [21]. The selectivity and affinity of the antagonists for H_3 receptor in those studies and conessine may be a key factor for the conflicting results. Conessine is more selective for H_3 receptor binding compared to other histamine receptors, specially H_4 receptor, than thioperamide or ciproxifan, drugs generally used as H_3 receptor antagonists [8]. For human H_4 receptor, for example, conessine pKi is < 5.00, while thioperamide has a pKi of 7.32 and ciproxifan of 5.73 [8]. Our drug also bind to the adrenergic alpha2C receptor, characteristic which may have influenced our results [8], considering the effects of alpha2 adrenoreceptor on drug-induced psychostimulation. Alpha2 adrenoreceptor blockade alters stimulant-induced psychostimulation [22]. Ethanol depressant effects are increased after acute treatment with blockers of norepinephrine transporter, and this effect is reversed by alpha2 adrenoreceptors antagonists [23]. The sensibility of the animals is also important in the analysis of ethanol-induced psychostimulation, due to the biphasic effects of this molecule on locomotion. In high doses, ethanol causes severe ataxia resulting in a decrease of locomotor activity [24,25]. Thereby, more susceptible animals would show decreased locomotor activity by H_3 receptor antagonist pretreatment. This effect would reflect an increase of ethanol effects, not a decrease as interpreted by others. Indeed, H_3 receptor antagonists increases ethanol induced loss of righting reflex in mice [26]. Interesting, H_3 receptor agonists also increase ethanol sedation effects [17,26]. In line with our results, thioperamide increases the psychostimulation of other abuse drug, cocaine [27–29].

CPP showed unexpected results. Conessine had no effect in the acquisition of ethanol induced CPP, while others showed blockade of ethanol CPP by pretreatment with H_3 antagonists [17,20,21]. H_3 receptor KO mice also did not show ethanol CPP [30]. Conversely, ciproxifan exacerbated ethanol CPP in 129/Sv mice. Different strains have different sensibility for ethanol CPP and probably the modulation of ethanol reinforcing proprieties is different across strains [31]. Thus, the use of various strains across the studies would be responsible for the contradictory results. Furthermore, in our study, conessine showed reinforcing proprieties *per se* on CPP procedure. To our knowledge, this effect has not yet observed for an H_3 antagonist and may have implications on the possible use of this antagonist on abuse drug related disorders. Focusing on ethanol induced CPP this reinforcing effect of conessine 10 mg/kg may have interfered in a potential effect of H_3 receptor blockade on ethanol induced CPP since it alone induce place preference. However, we tested also conessine on the dose of 1 mg/kg, which has no reinforcing effect *per se* and in spite of this did not block ethanol conditioning. Other studies testing

conessine effects the expression, extinction or reinstatement of ethanol-induced CPP may clarify this influence of H_3 receptors on ethanol positive reinforcement effect.

PFC is a brain area involved in information processing and decision-making. Its extensive connections with NAc make the PFC a key in the transition from the recreational to uncontrolled use of abuse drugs [5,32–35]. Several studies show neurochemical alterations in the PFC due to acute administration of ethanol and other abuse drugs [32,36,37]. Norepinephrine in PFC seems to be important for the reinforcing proprieties of drugs of abuse, whereas its depletion blocks stimulants, morphine and ethanol CPP [38–40]. In our study, animals treated only with ethanol or conessine showed a reduction of norepinephrine content in this brain area, both groups showing CPP for the drug paired site. Thus, norepinephrine reduction in the PFC may be related to CPP. Conversely, this alteration was not seen in animals treated both with conessine and ethanol. In this group, CPP may be related to the alterations on serotonin content and serotonergic turnover in the VTA. Indeed, there are some evidence of a role of the VTA $5HT_3$ receptors on ethanol reinforcing proprieties, being the antagonists of these receptors strong candidates for use in the treatment of alcoholism [41–44]. Dopaminergic turnover in the PFC was increased in vehicle/ethanol group, alteration that probably also influenced ethanol reinforcing proprieties.

Dopaminergic and noradrenergic neurotransmission in the NAc and CPu have an important role in the regulation of locomotor activity [45]. The alterations found on norepinephrine content and dopamine metabolites concentration in the CPu of ethanol treated groups would be related to the ethanol psychostimulation, whereas conessine exacerbation of ethanol induced-psychostimulation in conessine/ethanol group would be related to the decrease of dopamine metabolites in the SN.

In spite of possible influence of the neurochemical evaluations listed above with CPP or locomotor activity, it is difficult to establish a causal role. It is noteworthy that neurochemical evaluation by tissue punches as used in this work limits the interpretation of the results, since it does not differentiate intracellular from extracellular content of the neurotransmitters. Thus, results could not be interpreted as increase or decrease of neurotransmission but as increase or decrease of total tissue content for each molecule.

Therefore, conessine exacerbated ethanol-induced psychostimulation, with no effect on ethanol reinforcing proprieties in CPP paradigm. The highest conessine dose was reinforcing *per se*. Reinforcing proprieties of ethanol and conessine would be related with norepinephrine alterations in the PFC and serotonergic alterations in the VTA, while CPu dopaminergic and noradrenergic neurotransmission are possibly related to ethanol-induced psychomotor stimulation. Alterations in dopaminergic neurons of SN would be responsible for the exacerbation of ethanol-induced psychostimulation caused by conessine pretreatment.

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