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EFEITO DA TAURINA SOBRE O CONSUMO VOLUNTÁRIO DE ÁLCOOL E COMPORTAMENTOS DE RATOS

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Dissertação analisada e julgada adequada para obtenção do título de Mestre em Farmacologia e Terapêutica e aprovada em sua forma final pela Orientadora e pela Banca Examinadora.

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LISTA DE ABREVIATURAS, SÍMBOLOS E SIGLAS

BHE – barreira hematoencefálica

CEUA – Comissão de Ética no Uso de Animais

GABA – ácido γ-aminobutírico, do inglês *γ-aminobutyric acid*

HPA – eixo hipotálamo-pituitária-adrenal

IP – intraperitoneal

mGluR5 – receptores glutamatérgicos metabotrópicos do tipo 5

nAc – núcleo accumbens, do inglês *nucleus accumbens*

NMDA – receptor de glutamato N-metil-D-aspartato, do inglês *N-methyl-D-aspartate*

OFT – teste de campo aberto, do inglês *open field test*

PFC – córtex pré-frontal, do inglês *prefrontal cortex*

VTA – área tegmental ventral, do inglês *ventral tegmental area*

SNC– sistema nervoso central, do inglês *central nervous system*

RESUMO

Álcool é substância lícita de abuso, promovendo dependência pela modulação de sistemas neurotransmissores, como GABAérgico e dopaminérgico. Taurina, um aminoácido utilizado como suplemento alimentar e constituinte de bebidas energéticas, exerce efeito modulatório positivo sobre receptores $GABA_A$. Estudos em animais sugerem efeito terapêutico da taurina na síndrome de abstinência. No entanto, não se sabe o efeito da taurina sobre o comportamento de animais durante o uso de álcool. Portanto, o objetivo deste estudo foi avaliar o efeito do tratamento com taurina sobre o consumo voluntário de álcool e comportamentos de ratos. Foram oferecidas duas garrafas para ratos Wistar machos adultos, uma contendo álcool 20% em solução de sacarina 0,08% e a outra contendo apenas solução de sacarina 0,08% por 6 semanas. Ao grupo controle foram oferecidas duas garrafas contendo sacarina 0,08%. O consumo diário de líquidos dos dois grupos foi monitorado. No 22º dia eles foram divididos em 4 grupos (n=12/grupo) para receber taurina 100 mg/kg (grupos Álcool/TAU e Controle/TAU), via intraperitoneal, uma vez ao dia, por 19 dias, ou solução salina 0,9% (grupos Álcool/SAL e Controle/SAL). Nos dias 22 e 33, os ratos foram expostos ao teste de campo aberto e, no dia 34, ao teste de claro/escuro. Nossos resultados mostraram que o tratamento com taurina aumentou em mais de 10% a preferência por álcool em relação aos animais não tratados. O consumo de álcool médio no grupo Álcool/SAL foi 12 g/kg/dia, enquanto que no grupo Álcool/TAU foi 20 g/kg/dia, sendo que taurina aumentou significativamente o consumo a partir o sexto dia de tratamento. No campo aberto, dose aguda de taurina reduziu a ambulação total de animais controle, além de aumentar os cruzamentos centrais do grupo álcool. Taurina aguda e crônica aumentou a latência de auto-limpeza (*grooming*) e apenas o tratamento crônico reduziu

a frequência de *grooming* independentemente dos grupos. No teste de claro/escuro, taurina aumentou o tempo de permanência no compartimento claro, o número de transições entre os compartimentos e a latência para entrar no compartimento escuro apenas no grupo álcool, indicando efeito tipo-ansiolítico. Conclui-se que o tratamento crônico com taurina aumenta o consumo voluntário e preferência por álcool em ratos, possivelmente por efeito sinérgico com o álcool que facilita a ativação da via de recompensa dopaminérgica. O efeito tipo-ansiolítico da taurina no grupo álcool também pode ser justificado por efeito aditivo sobre receptores $GABA_A$. Demonstramos que a taurina não apresenta efeito antiaditivo e, talvez, como o acamprosato, possa ser efetiva no controle da recaída, somente após abstinência.

Palavras-chave: Etanol, dependência de drogas, bebidas energéticas, consumo voluntário

ABSTRACT

Alcohol is a licit substance of abuse, promoting dependence by the modulation of different neurotransmitter systems, such as GABAergic and dopaminergic. Taurine, an amino acid used as food supplement and constituent of energy drinks, exerts positive modulatory effect on $GABA_A$ receptors. Animal studies suggest a therapeutic effect of taurine on withdrawal alcohol syndrome. However, the effect of taurine on the behavior of animals during alcohol use is not known. Therefore, the aim of this study was to evaluate the effect of taurine treatment on voluntary alcohol consumption and on behaviors of rats. Two bottles were offered to adult male Wistar rats, one containing 20% alcohol in 0.08% saccharin solution and another containing only 0.08% saccharin solution for 6 weeks. Two bottles containing 0.08% saccharin were offered to the control group. The daily liquid intake of both groups was monitored. On day 22 they were divided into 4 groups (n =12/group) to receive taurine 100 mg/kg (Alcohol/TAU and Control/TAU groups), intraperitoneally, once a day, for 19 days, or 0.9% saline (Alcohol/SAL and Control/SAL groups). On days 22 and 33, rats were exposed to the open field test and, on day 34, to the light/dark test. Our results showed that taurine treatment increased by more than 10% the alcohol preference compared to untreated animals. The average alcohol consumption in the Alcohol/SAL group was 12 g/kg/day, while in the Alcohol/TAU group it was 20 g/kg/day, and taurine significantly increased the consumption from the day 6 of treatment. In the open field, acute dose of taurine reduced the total ambulation of control animals and increased central crossings of alcohol group. Acute and chronic taurine increased the latency for grooming, and only chronic treatment reduced the frequency of grooming independently of the groups. On the light/dark test, taurine increased the time spent in light compartment, the number of

transitions between compartments and the latency to enter the dark compartment only in the alcohol group, indicating an anxiolytic-like effect. It is concluded that chronic taurine treatment increases alcohol voluntary consumption and preference in rats, possibly due to synergistic effects with alcohol that facilitates the activation of the dopaminergic reward pathway. The anxiolytic-like effect of taurine in the alcohol group may also be justified by additive effect on $GABA_A$ receptors. Our results show that taurine has no anti-additive effect and, perhaps as acamprosate, may be effective in controlling relapse only after abstinence.

Keywords: Ethanol, drug addiction, energetic beverages, voluntary consumption

1. REVISÃO DA LITERATURA

1.1 Consumo de álcool e suas implicações

O álcool está entre as substâncias lícitas de abuso mais utilizadas no mundo, mesmo havendo diversas políticas públicas para redução do seu consumo (Meyerhoff et al., 2006). Seu uso abusivo representa a 3ª maior causa de doenças nos países desenvolvidos e a 5ª causa de morte prematura e de incapacidade no mundo todo, totalizando cerca de 4% da mortalidade mundial (OMS, 2014). É considerado fator causal de mais de 200 doenças, sendo mais prevalentes as doenças cardiovasculares e diabete (33%), doenças gastrintestinais (16%), cânceres (12%), doenças neuropsiquiátricas, além de acidentes de trânsito (OMS, 2014).

Após ingestão por via oral, o álcool é rapidamente absorvido pelo trato gastrintestinal, distribuído para diversos tecidos e excretado principalmente após metabolização por enzimas hepáticas. Por ser uma molécula pequena, de baixo peso molecular, e encontrar-se na forma não-ionizada em pH fisiológico, o etanol atravessa facilmente membranas biológicas por difusão, inclusive a barreira hematoencefálica (BHE), atingindo o sistema nervoso central (SNC) (Mullen, 1977). Ali, na dependência da dose, atua sobre diferentes sistemas neurotransmissores, apresentando efeito ansiolítico, sedativo e relaxante da musculatura. Também promove euforia, agitação, incoordenação motora e, em longo prazo, déficit cognitivo e de memória (McKeon et al., 2008). Quando usado crônica e abusivamente, promove dependência (alcoolismo), que é considerada uma doença crônica recorrente caracterizada por episódios frequentes de intoxicação; uso, apesar de consequências adversas; compulsão; perda do controle sobre o uso; e sintomas emocionais negativos durante abstinência (APA, 1994).

1.2 Neurobiologia do alcoolismo

O álcool possui múltiplos alvos de ação que convergem para a ativação de vias neurais de recompensa, promovendo dependência (Gilpin e Koob, 2008). A via mais estudada neste contexto é a mesolímbica dopaminérgica. Esta via, que projeta neurônios dopaminérgicos da área tegmental ventral (VTA) até o núcleo accumbens (nAc) e córtex pré-frontal (PCF) é um dos mais importantes substratos neurais para a geração de estímulos recompensatórios provocados por drogas de abuso, inclusive o álcool (Nestler, 2005). Cada droga, apesar de mecanismo de ação distintos, parece aumentar direta ou indiretamente a transmissão dopaminérgica nesta via. O álcool por sua vez, atua importantemente como modulador positivo de receptores de ácido γ-aminobutírico (GABA) do tipo A. Na área tegmental ventral, a ativação de receptores $GABA_A$ aumenta a liberação de dopamina no núcleo accumbens, ativando processos de recompensa (Gilpin e Koob, 2008).

Adicionalmente, álcool atua como modulador negativo glutamatérgico principalmente sobre receptores de N-metil-D-aspartato (NMDA) e receptores glutamatérgicos metabotrópicos do tipo 5 (mGluR5) cerebrais, inibindo a liberação de glutamato dos terminais nervosos que agem nos neurônios do nAc (Gilpin e Koob, 2008; Roberto e Varodayan, 2017). Além destes, outros mecanismos são propostos pelos quais o álcool pode atuar nestas vias de recompensa, incluindo a modulação dos sistemas opioide, endocanabinoide ou até mesmo diretamente em neurônios dopaminérgicos (Brodie et al.; Erdozain e Callado, 2011).

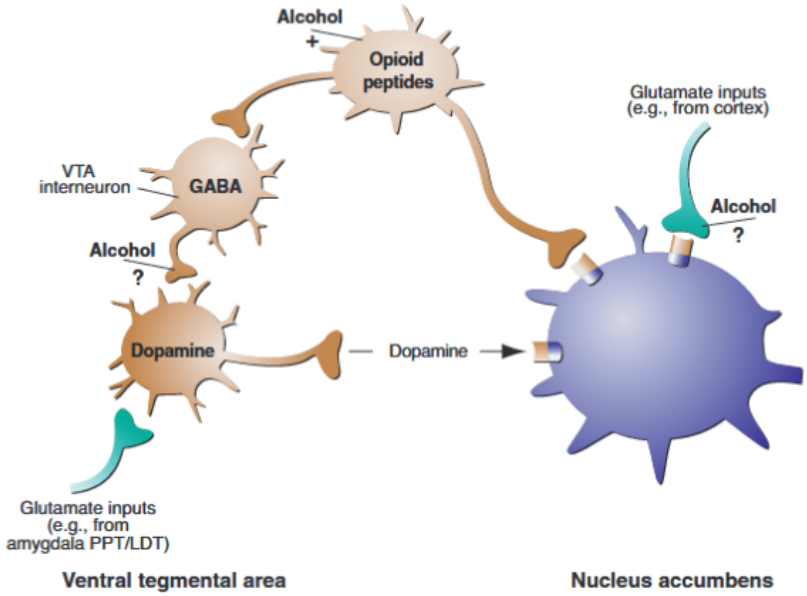

Figura 1: Efeitos do álcool em sistemas neurotransmissores envolvidos em vias neurais de recompensa (Gilpin e Koob, 2008).

Com o uso continuado de altas doses de álcool observam-se processos neuroadaptativos na função neuronal de diversos sistemas neurotransmissores que afetam a motivação pelo uso e contribuem para o estabelecimento e manutenção da dependência, resultando em sensibilização e tolerância aos efeitos prazerosos (Gilpin e Koob, 2008; Roberto e Varodayan, 2017). Sensibilização refere-se à intensificação do valor reforçador positivo do álcool após exposição repetida, enquanto tolerância refere-se à redução do valor reforçador do álcool após repetição das administrações, sendo assim necessárias maiores doses para atingir efeito prazeroso (Gilpin e Koob, 2008). Uma vez ocorridos estes processos adaptativos, a retirada do álcool leva à síndrome de abstinência, cujos sinais e sintomas parecem estar relacionados à ativação de vias glutamatérgicas.

Síndrome de abstinência de álcool constitui um conjunto de sinais e sintomas que iniciam geralmente nas primeiras 24 a 48 horas após a última ingestão. Entre os sintomas mais frequentes em humanos estão ansiedade, anedonia, delírio, agitação,

irritabilidade e convulsões (McKeon et al., 2008). Em roedores, esta síndrome se manifesta por ansiedade, diminuição da interação social, hipolocomoção, redução da ingesta de alimento palatável e da busca por novidades (Fukushiro et al., 2012; Kliethermes, 2005; Overstreet et al., 2002; Slawecki e Roth, 2004). Esta fase da dependência de álcool é considerada crítica para a recaída e manutenção do ciclo da adição, devido à geração de sintomas físicos e neuropsicológicos (Koob e Le Moal, 2001). Abstinência de álcool, além de alterações comportamentais, promove alterações neuroquímicas no SNC, afetando os sistemas dopaminérgico (Koob e Le Moal, 2001), GABAérgico (Tsai e Coyle, 1998), glutamatérgico (Kalivas, 2009; Tsai e Coyle, 1998) e de endocanabinóide (Erdozain e Callado, 2011), além de afetar a regulação do eixo hipotálamo-pituitária-adrenal (HPA) e promover mudanças na concentração de peptídeos relacionados ao apetite (Aguiar-Nemer et al., 2013). Associado a essas alterações, uso crônico de álcool ou sua abstinência aumentam resposta de estresse oxidativo e neuroinflamatória por liberação de proteínas pró-inflamatórias em diversas áreas encefálicas (Kelley e Dantzer, 2011). Todos estes processos contribuem para dano e morte neuronal, com consequências graves à cognição e qualidade de vida dos usuários.

1.3 Tratamento da dependência de álcool

Poucos recursos farmacológicos estão disponíveis para controle dos sinais de abstinência e compulsão para indivíduos que decidem interromper o uso do álcool. Dissulfiram, naltrexona e acamprosato são alguns fármacos utilizados para redução dos efeitos reforçadores do álcool ou dos sinais de abstinência (Gilpin e Koob, 2008). No entanto, alguns alcoolistas não respondem bem a esses tratamentos, tornando necessário o estudo de alternativas terapêuticas que possam auxiliar na interrupção ou redução do

consumo diário de álcool para concentrações seguras, reduzindo também os riscos associados ao abuso.

1.4 Taurina

Taurina (ácido 2-aminoetanosulfônico) é um β-amino ácido intracelular, semi-essencial, não incorporado em proteínas que está presente abundantemente no SNC (Huxtable, 1992). Participa do controle de diversas funções fisiológicas, exercendo principalmente propriedades osmorregulatória, citoprotetora, neuromodulatória, antioxidante e anti-inflamatória (Gu et al., 2015; Huxtable, 1992; Rosemberg et al., 2010). Durante sua biossíntese, que ocorre a partir da ingestão de fontes de enxofre reduzido na forma de cisteína e metionina, é consumido um radical hidroxil – o que intensifica sua capacidade antioxidante (Huxtable, 1992). Fígado é o local principal de síntese e armazenamento de taurina, de onde é distribuída para outros órgãos por transporte ativo por meio do seu transportador específico, a proteína Tau-T, que utiliza gradiente transmembrana de íons sódio e cloreto como força motriz (Huxtable, 1992; Kozlowski et al., 2008). Desse modo, uma administração sistêmica de taurina atingirá o cérebro por meio deste carreador saturável, transportando-a do sangue até o interior da célula endotelial.

No SNC, taurina exerce importante função neuroprotetora contra excitotoxicidade neuronal induzida por glutamato, agindo como modulador negativo glutamatérgico, tanto diretamente como um antagonista parcial de receptores NMDA, como indiretamente na regulação da homeostase de cálcio intracelular, reduzindo o influxo de cálcio após ativação continuada do sistema (Chan et al., 2014; Wu et al., 2005). Taurina é um análogo estrutural do neurotransmissor GABA, atuando como modulador positivo de receptores $GABA_A$ e de glicina (Albrecht e Schousboe, 2005).

Assim, taurina estimula o influxo de íons cloreto em neurônios pós-sinápticos, hiperpolarizando membranas por meio de diversos mecanismos, podendo agir diretamente abrindo canais de cloreto ou indiretamente (Albrecht e Schousboe, 2005). Estudos sugerem a existência de um receptor putativo específico para taurina, o qual atuaria também na modulação do transporte de íons cloreto (Frosini et al., 2003b, 2003a).

1.5 Interações entre taurina e álcool

A liberação de taurina é aumentada e sua captação é inibida em condições de dano celular, de modo que o aumento extracelular deste aminoácido parece ser um importante mecanismo de proteção endógeno (Gu et al., 2015; Saransaari e Oja, 2000). Desta forma, taurina pode atuar restaurando danos induzidos pelo álcool, tanto no SNC, prevenindo o aumento de parâmetros de estresse oxidativo (Rosemberg et al., 2010), como perifericamente, protegendo contra danos teciduais induzidos pelo consumo crônico de álcool nos tecidos adiposo e hepático (Chen et al., 2009). De fato, um aumento da taurina extracelular em resposta a administração de álcool já foi observado em diversas regiões encefálicas de ratos (Quertemont e Grant, 2004; Quertemont et al., 1999).

Recentemente foi demonstrado que taurina restaura comportamentos exploratórios alterados pela abstinência de álcool em ratos (Hansen et al., 2017), e que a sua concentração endógena extracelular no nAc pode controlar o pico dopaminérgico provocado pela administração aguda de álcool nesta área encefálica, sugerindo que este amino ácido pode exercer um importante papel em vias neurais de recompensa (Ericson et al., 2011). Além disso, acamprosato, um derivado sintético da taurina foi aprovado

para tratamento da síndrome de abstinência ao álcool, porém é um fármaco de alto custo e de difícil acesso no Brasil.

Estudos sugerem que a modulação do sistema GABAérgico pode ser importante alvo de fármacos que atenuem os efeitos reforçadores positivos causados por drogas de abuso como o álcool, auxiliando na redução do consumo (Brebner et al., 2002). De fato, pré-tratamento agudo com taurina reduziu o consumo agudo de álcool em ratos, sem afetar a preferência pelo álcool (Olive, 2002), assim como pré-tratamento com diferentes doses de taurina preveniu alterações comportamentais provocadas pela exposição aguda a etanol em peixes, resultando em efeito bifásico dependente da dose (Rosemberg et al., 2012). No entanto, o possível efeito antiaditivo de doses repetidas de taurina sobre o consumo voluntário crônico de álcool ainda não foi explorado.

2. HIPÓTESE

Considerando que taurina é uma substância com múltiplos alvos de ação, capaz de modular tanto o sistema GABAérgico como glutamatérgico, nossa hipótese é que o tratamento crônico com taurina poderia restaurar o equilíbrio entre excitação e inibição desses sistemas que estãoe alterados pelo uso crônico de álcool, e assim reduzindo o consumo voluntário de álcool em ratos.

3. OBJETIVOS

2.1 Objetivo Geral

Avaliar o efeito de doses repetidas de taurina sobre o consumo voluntário crônico de álcool em ratos, bem como seus efeitos agudos e crônicos sobre parâmetros comportamentais.

2.2 Objetivos específicos

- Avaliar o efeito antiaditivo da taurina, determinado pelo consumo voluntário em ratos dependentes de álcool;
- Avaliar comportamento ambulatório e de ansiedade em ratos dependentes de álcool tratados aguda e cronicamente com taurina no teste de campo aberto;
- Avaliar comportamento de ansiedade em ratos dependentes de álcool tratados cronicamente com taurina no teste de claro/escuro.

Os resultados deste estudo foram organizados na forma de um artigo científico para submissão à revista *Pharmacology Biochemistry and Behavior*.

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4. ARTIGO CIENTÍFICO

Taurine enhances voluntary alcohol intake and promotes anxiolytic-like behaviors in rats

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ABSTRACT

Taurine is an amino acid with relevant physiologic and pharmacologic functions, usually used as a food supplement and added to energetic drinks. Acute taurine administration decreases the alcohol voluntary intake and sub chronic treatment restores the exploratory behaviors impaired by alcohol withdrawal in rodents. Because there are no results for chronic taurine and alcohol treatment, we aimed to evaluate the effects of chronic taurine treatment on alcohol voluntary consumption and on behavioral parameters in rats. Adult Wistar rats were shared in two groups and made free to choose from two bottles, containing alcohol (20%, AL group) or saccharin (0.08%) solution, or two bottles containing saccharin solution (CT group), 24h/day for 6 weeks. After 3 weeks, rats were divided to receive 100 mg/kg taurine (TAU) or saline (SAL), intraperitoneally, once a day, for 3 weeks. Rats were exposed to the open field test on day 22 after an acute taurine administration, and on day 33 after chronic treatment. On day 34 rats were exposed to the light/dark test. Curiously, taurine treated rats increased the alcohol preference by 13%, and the alcohol intake by almost double compared with SAL rats. Acute taurine decreased total ambulation in CT group and increased central crossings in AL group. Chronic taurine treatment increased the time spent in light compartment only in the AL group. Taurine enhanced the voluntary alcohol intake and preference in rats, and produces anxiolytic-like behavior only in alcohol treated rats, probably due to synergistic effect with alcohol on dopaminergic and GABAergic systems, respectively.

Keywords: Ethanol, drug addiction, energetic beverages, voluntary consumption

1. INTRODUCTION

Alcohol abuse is a serious public health problem, representing the third largest cause of illness in developed countries and the fifth leading cause of premature death and disability worldwide, accounting for about 4% of global mortality (WHO, 2014). Alcohol exerts positive modulatory effect on γ-aminobutyric acid $(GABA)_A$ receptors and acts as a negative glutamatergic modulator (Gilpin and Koob, 2008; Roberto and Varodayan, 2017). Besides these, many others mechanisms are proposed by which alcohol may indirectly activate dopamine in the mesolimbic reward pathway, including modulation of endogenous opioid and endocannabinoid systems, or it can even act directly on dopaminergic neurons of these brain areas (Brodie et al.; Erdozain and Callado, 2011).

Chronic use of high amounts of alcohol, however, causes neuro-adaptive processes that alters the functionality of the reward system, resulting in a blunted dopamine transmission that affects the motivation for alcohol use and leads to addiction (Gilpin and Koob, 2008; Martinez et al., 2005; Roberto and Varodayan, 2017). Abrupt withdrawal of alcohol, on this condition, causes the abstinence syndrome, whose unpleasant symptoms appear to be related to the activation of glutamatergic pathways and are often responsible for relapses in individuals who decide to stop drinking (McKeon et al., 2008). In fact, resulted alterations in the activity of dopaminergic neurons of ventral tegmental area (VTA), correlated with extracellular levels of dopamine in nucleus accumbens (nAc), are involved in the establishment of drug dependence as well as susceptibility to relapse (Marinelli et al., 2003).

Taurine (2-aminoetanesulfonic acid), one of the constituents of energetic drinks, is an intracellular β-amino acid found highly abundant in central nervous system (CNS) (Huxtable, 1992). It plays a role in several physiological processes, exerting

osmoregulatory, anti-inflammatory and antioxidant functions (Gu et al., 2015; Huxtable, 1992; Rosemberg et al., 2010). Taurine exerts neuroprotection against glutamate induced neuronal excitotoxicity, acting as a partial antagonist of N-methyl-D-aspartate (NMDA) receptors or indirectly regulating the intracellular calcium homeostasis (Chan et al., 2014; Wu et al., 2005). Taurine stimulates the influx of chloride ions into post-synaptic neurons by positive modulatory effect on $GABA_A$ and glycine receptors or by directly opening chloride channels (Albrecht and Schousboe, 2005), or maybe even by modulation of a putative specific taurine receptor (Frosini et al., 2003b, 2003a).

Taurine has been shown to restore alcohol-induced damage by preventing oxidative stress in CNS (Rosemberg et al., 2010). In fact, an increase in extracellular taurine levels in response to alcohol administration has been observed in several brain regions of rats (Olive, 2002; Quertemont and Grant, 2004; Quertemont et al., 1999). Taurine pretreatment altered the behavior produced by acute exposure to ethanol in zebrafish and mice, resulting in an effect depending on the alcohol and taurine dosages (Aragon et al., 1992; Rosemberg et al., 2012). Even though other study did not observe this interactions between taurine pretreatment and alcohol in mice (Ginsburg and Lamb, 2008). Taurine seemed to restore the exploratory behavior altered by alcohol withdrawal in rats (Hansen et al., 2017), and its extracellular concentration in nAc may modulate the dopaminergic peak caused by acute alcohol administration in this brain area (Ericson et al., 2011), suggesting an important role in neural pathways of reward.

Taurine could restore the imbalance between excitation and inhibition (El Idrissi and Trenkner, 2004) caused by chronic alcohol use in GABAergic and glutamatergic systems. A taurine synthetic derivative, acamprosate, has already been approved for alcoholism treatment. Modulation of GABAergic system may be an important target for drugs that attenuate the positive reinforcing effects caused by drugs of abuse such as

alcohol, helping to reduce consumption (Brebner et al., 2002). In fact, acute taurine pretreatment reduced the acute alcohol consumption in rats (Olive, 2002). However, the effect of repeated doses of taurine on chronic alcohol consumption has not yet been explored. We hypothesize that taurine, because its multiple targets could reduce alcohol consumption in alcoholic rats. Thus, the aim of this study was to evaluate the effect of taurine treatment on alcohol voluntary consumption in rats and its effect on behavioral tests.

2. MATERIALS AND METHODS

2.1 Animals

Male Wistar adults rats (~270 g) were kept under a 12 h light/dark cycle (lights on at 7:00 AM) at 22 ± 1°C and 55% air humidity in polypropylene cages (40 x 33 x 17.8 cm) divided by perforated aluminum grid, insurmountable, fixed longitudinally (n = 2/box) allowing free access to solutions and standard rat chow. All protocols followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethical Committee of the Federal University of Rio Grande do Sul (CEUA-UFRGS #32850).

2.2 Chemicals

Saccharin sodium dihydrate (Sigma-Aldrich, MO, USA) was dissolved in tap water at the concentration of 0.08% (w/v). Ethanol 99% (Merck, São Paulo, BR) was diluted at 20% (w/v) in saccharin solution 0.08%. Taurine (Sigma, St. Louis, MO, USA) was dissolved in saline solution (NaCl 0.9%) at the concentration of 100 mg/mL, and each animal was administered intraperitoneally (IP) with a volume of 1 mL/kg.

2.3 Alcohol voluntary consumption protocol

After 7 days of environmental habituation, the animals were initially divided into two groups (n = 24/group): Control and Alcohol. To the Control group of animals were offered 2 bottles containing saccharin solution for self-administration. At the same way, to the Alcohol group was offered a bottle containing saccharin and another containing alcohol solution with saccharin (Sabino et al., 2013). These sweet, highly palatable, non-caloric solutions were offered to the animals to stimulate voluntary consumption. Consume of liquids were measured daily, at 2:30 PM, by the difference of weight of the bottles from the amount added on the previous day. Immediately after, bottles were refilled and replaced. To avoid the development of place conditioning preference, the side of each bottle on the cage was alternated daily. Rats were weighted weekly, in order to determine daily ethanol consumption relative to body weight (g/kg) and to adjust taurine dosages. This protocol was followed throughout the experimental period. Total volume of fluid consumed per day was obtained by the sum of the consumption of both bottles offered to Control and Alcohol groups, regardless of whether the solution was saccharin or alcohol. Ethanol preference was calculated by the ratio between the total amount of ethanol consumed and the total of both liquids consumed (ethanol + saccharin bottles) × 100.

2.4 Taurine treatment

On day 22, Control and Alcohol groups were subdivided (n = 12/group) into: Control/SAL or Alcohol/SAL groups, administrated with 1 mL/kg saline IP and Control/TAU or Alcohol/TAU groups, administrated with 100 mg/kg taurine IP. These administrations were performed daily, once a day at 6:00 PM – 60 min before dark

cycle – for 19 days. The taurine dose was chosen based on appearance of antidepressant effects in rats (Caletti et al., 2015). Consume of chow was weekly measured, as well as rats body weight, to observe possible variations on feeding behavior caused by taurine treatment.

2.5 Behavioral tasks

Animals were habituated to the test room for two hours and tasks were performed between 2:00 PM to 5:00 PM.

2.5.1 Open field task

On the day 22 – 60 min after the first taurine administration – and on the day 33 – 24 h after taurine administration from the previous day – rats were individually placed in the center of a white square arena (80x80x40 cm), which floor was divided into 16 squares (Hansen et al., 2017). The test was performed in a quiet room, illuminated by a dim light (25 W), and was recorded by a video camera for 6 min. The number of squares crossed with four paws (central and peripheral crossings), rearing responses, grooming responses, the latency for the first grooming, the latency for center output and the number of fecal boli were evaluated in the recorded video by a trained and blinded observer using Wabehav software (Wabehav, Boston, USA). The apparatus was cleaned with ethanol 10% between each rat to remove any trace of odor.

2.5.2 Light/dark task

The light/dark apparatus consisted of a rectangular box with two separated compartments. The dark compartment had black walls, floor, and ceiling, without illumination (35×41×21 cm). The light chamber, illuminated by a 100 W lamp placed

above the center of the box, had white walls and floor, and transparent ceiling (45×41×21 cm). Between these two compartments there was an opening door (5×8 cm), giving to the animals free access to both sides of the box (Almeida et al., 2010). On day 34 – 24 h after last taurine administration – rats were individually placed in the light compartment facing away from the opening door and allowed to freely explore the apparatus for 5 min recorded by a video camera. The light/dark apparatus was cleaned with ethanol 10% and dried between each animal. The time spent in the light compartment, the number of transitions between compartments, the latency time to enter for the first time in the dark compartment and risk assessment behavior were evaluated in the recorded video by a trained and blinded observer using Wabehav software (Wabehav, Boston, USA).

2.6 Statistical analyses

Data were tested for normal distribution by the Kolmogorov-Smirnoff test. For temporal evaluation of alcohol, vehicle, and chow consumption, body weight gain, and alcohol preference along the experiment we performed a 2-way repeated measures (RM) analysis of variance (2-way RM-ANOVA), followed by Bonferroni's multiple comparison *post hoc* test when applicable. Behavioral parameters and global analyses of consumption were analyzed by a 2-way analysis of variance (2-way ANOVA) using as factors the condition (control or alcohol) and treatment (saline or taurine), followed by Bonferroni's test. A level of $P < 0.05$ was set to consider statistical significance. Data were expressed as the means ± SEM. Statistical analyses were performed using Sigma Plot® 12.0 (Systat Software Inc. San Jose, CA, USA).

3. RESULTS

3.1 Total fluid intake, alcohol consumed and preference

Evaluation of total volume of fluids consumed throughout the experiment showed that Control animals drank on average about 60 mL of liquids per day, while this volume consumed by Alcohol group was 1.5 times higher ($F_{(3,96)}$ = 14.90; P < 0.001) (Data not shown). Taurine treatment did not interfere on the total volume of fluids consumed, regardless of the group to which the animals belonged.

Alcohol/SAL animals preferred to drink alcohol 32% more than saccharin solution. However, taurine significantly increased in 13% the preference for alcohol ($F_{(1,45)}$ = 7.83; P < 0.005) (Figure 1). Two way repeated measures ANOVA showed a significant interaction between Alcohol/TAU group and days, demonstrating that taurine injections enhanced alcohol preference from the beginning of the treatment according to the days ($F_{(19,450)}$ = 1.809; P < 0.020).

Even with the increase in alcohol preference already on the first day of treatment, the amount of alcohol consumed only starts to enhance significantly six days after the beginning of injections in Alcohol/TAU group in comparison with Alcohol/SAL group ($F_{(19,438)}$ = 10.89; P < 0.001), remaining increased during subsequent days ($F_{(19,438)}$ = 1.62; P < 0.049) (Figure 2). The average alcohol consumption in Alcohol/SAL group was 12 g/kg/day, while in the Alcohol/TAU group was 20 g/kg/day, representing an increase of almost double the alcohol consumption produced by treatment with taurine ($F_{(1,438)}$ = 4.89; P < 0.038) (Figure 2).

Neither alcohol nor taurine *per se* significantly changed the rat's body weight gain or the chow consumption by any group of animals along the weeks (Data not shown).

3.2 Behavioral tasks

3.2.1 Open field tasks

On the first open field exposure, a single acute dose of taurine decreased the total ambulation only in the Control/TAU group ($F_{(1,41)}$ = 8.204; P = 0.023) (Figure 3-A), while peripheral crossings were decreased both in Control/TAU ($F_{(1,44)}$ = 15.890; P = 0.004) and Alcohol/TAU groups compared to their respective SAL groups ($F_{(1,44)}$ = 15.890; P = 0.014) (Figure 3-B). Central crossings, in turn, were increased only in Alcohol/TAU group ($F_{(1,44)}$ = 8.78; P = 0.013) (Figure 3-C). Frequency of rearing was significantly decreased in Alcohol/TAU group ($F_{(1,42)}$ = 5.64; P=0.024) (Figure 3-G), as well as the number of fecal boli ($F_{(1,47)}$ = 4.74; P = 0.026; $F_{(1,47)}$ = 6.45; P = 0.014) (Figure 3-H). No difference was observed in the latency of center output and grooming behavior parameters. Taurine treatment increased the latency for the first grooming, regardless of whether alcohol or just vehicle bottles have been offered to them, both on the first ($F_{(1,43)}$ = 4.526; P = 0.040) (Figure 3-E) and on the second open field test ($F_{(1,43)}$ = 5.54; P = 0.024) (Figure 4-E). On the other hand, on second open field exposure, grooming frequency was also decreased in these same groups ($F_{(1,43)}$ = 4.145; P = 0.048) (Figure 4-F). No significant changes were observed between the groups in others parameters analyzed in the second open field task.

3.2.2 Light/dark task

On the light/dark test, only in alcohol animals, taurine produced a significant increase in the time spent in the light compartment ($F_{(1,42)}$ = 10.49; P = 0.008) (Figure 5-A), in the number of transitions between light and dark compartments ($F_{(1,42)}$ = 9.11; P = 0.019) (Figure 5-B), and in the latency time to enter for the first time in the dark compartment compared with Alcohol/SAL group ($F_{(1,42)}$ = 7.434; P = 0.016) (Figure 5-

C). However, none significant changes were observed in these parameters between the other groups or in the frequency of risk assessment behavior (Figure 5-D).

4. DISCUSSION

Conversely to what we expected, taurine treatment significantly enhanced the voluntary alcohol intake and preference in rats. Acute and chronic taurine also produced an anxiolytic-like behavior only in alcohol treated rats. Previously, it has been shown that taurine given as an acute pretreatment reduced the alcohol voluntary consumption in rats (Olive, 2002), as well as taurine treatment prevents some behavioral changes of alcohol withdrawal in rats (Hansen et al., 2017). However, the combined effect of a simultaneous alcohol chronic exposure and repetitive taurine dosages has been firstly demonstrated here.

Alcohol, through various mechanisms of action, is capable of activating reward neural pathways, such as the mesolimbic dopaminergic pathway, promoting the release of dopamine in nAc (Gilpin and Koob, 2008). Increased dopamine in this brain area is related to pleasurable sensations and is one factor to the intensification of positive reinforcement values of drugs of abuse, such as alcohol (Gilpin and Koob, 2008). In animal models of addiction, the activation of the reward pathway could be inferred by the voluntary intake of high amounts of drugs or even by a conditional place preference test (Quertemont et al., 1998). Here we observed the continued voluntary daily consumption of high ethanol doses, suggesting that this pathway has been activated.

It has been shown that alcohol administration increases extracellular taurine levels in different brain areas, and that could represents an endogenous protective mechanism against alcohol neurotoxicity (Ericson et al., 2011; Olive, 2002; Quertemont et al., 1999). A study using the microdialysis technique showed an increase on taurine

extracellular levels in the nAc of rats as a result of the direct alcohol administration in this brain area, accompanied by the expected ethanol-induced dopaminergic local peak (Ericson et al., 2011). However, when taurine was supplemented in association with the local ethanol administration the resulted dopaminergic peak occurs faster, as well as they observed that the presence of a taurine extracellular peak obtained as a consequence of alcohol administration or even by supplementation seemed to be necessary to the ethanol-induced dopaminergic increase in nAc occurs (Ericson et al., 2011). Moreover, it has been shown by the same technique that the acute alcohol voluntary consumption results in a very large increase of extracellular endogenous taurine and dopamine levels in nAc of rats (Ericson et al., 2011, 2017). Chronic voluntary consumption of approximately 6 g/kg/day of ethanol, in turn, causes only a slight increase in these taurine and dopamine amounts, suggesting neuro-adaptative disturbance by chronic alcohol use (Ericson et al., 2017). Considering that our model of voluntary intake promoted a chronic consumption of even larger amounts of alcohol, we can infer that taurine treatment could be improving the alcohol-induced release of dopamine in the nAc, activating reward and reinforcement processes faster and more efficiently than alcohol alone. Therefore, it is possible that taurine-treated rats drank more alcohol because they obtained more pleasure/reward than untreated animals.

Taurine may thus have acted by the restoration of dopaminergic neuroadaptations induced by chronic alcohol use or also by a synergistic effect with alcohol in this system, since the administration of taurine alone on nAc does not seems to directly promote dopaminergic peak in this brain area (Ericson et al., 2011). Moreover, a study has demonstrated that rats supplemented with oral taurine showed a decrease in aversion caused by high doses of alcohol (Quertemont et al., 1998). Therefore, taurine may also participate to physiological adaptations produced by the

chronic consumption of high amounts of ethanol, reducing the undesirable effects of alcohol and thus enhancing its consumption.

In our study, the average alcohol consumption of both groups during all experimental period was about 15 g/kg/day, representing more than twice the consumption obtained in a 2-bottle-choice voluntary model in which the control solution was water (Ericson et al., 2017). Thus, it may be suggested that the palatability produced by saccharin is one factor that contributes with this unpredictable high alcohol consumption. Besides that, our experimental model allowed the rats to consume alcohol freely for 24 h, including at night when they are most active, unlike most of others experiments of voluntary intake that limit alcohol access to few hours by day (Carnicella et al., 2014; Planeta, 2013). Additionally, to avoid social isolation our rats were keep in pairs on cages, which can also influences this parameter. Saccharin is often used in animal models of addiction to stimulate the drug's voluntary intake, because it is a non-caloric sweetener. Recently, it has been shown that a percentage of rats that exerts high alcohol preference maintain it even instead of high sweetness reward saccharin solutions (Augier et al., 2018). Studies have shown that the reinforcement value obtained by saccharin is not related to the activation of dopaminergic reward pathways, and suggest that the nutritional value is critical for dopamine mediated reward even for palatable substances (Beeler et al., 2012; Cannon and Palmiter, 2003). Anyway, here saccharin was present in both bottles at the same concentration to avoid its possible influence on solutions preference. Considering only control animals, the daily total volume of fluids intake (saccharin+saccharin bottles) were also about twice higher than regular hydric intake by Wistar rats under standard conditions (Fox, 2015). However, the possibility of choosing to drink alcohol increased even more the total volume of fluids intake (saccharin+alcohol bottles) in alcoholic groups, representing almost double

of daily liquid consumption of controls, with about 40% preference for drinking in the alcohol bottle. Moreover, the hydric consumption of alcohol groups was similar to control groups, evidencing that the search for alcohol was voluntary and did not depend on dehydration. Taurine treatment also does not interfere in total fluids intake, neither in body weight gain or food intake by any group of rats (Data not shown). Therefore, it may be inferred that its outcome on the increase in alcohol consumption was not an effect of a generalized fluid intake or of feeding alterations.

A single acute dose of taurine decreased ambulation only in control animals, while peripheral crossings were decreased in both control and alcohol treated groups. However, there was a significant increase in central crossings in Alcohol/TAU group, indicating that the total ambulation of these animals was not affected since they stated to ambulate more in the central area. Ambulation of this same group was also not significantly changed by chronic taurine treatment on the second open field exposure. Although the open field is not a specific test for anxiety assessment, when animals walks more in the center of the apparatus it may be suggested that the new environment is producing less anxiety to them (Prut and Belzung, 2003). A reduction on the number of fecal boli may be also related to less anxiety, as we observed here on the same group of rats. Once light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on their spontaneous behavior in response to mild stressors, its results can be better interpreted as measures of anxiety (Bourin, 2015). Here we observed an enhancement of anxiolytic-like behavior on light/dark box only in Alcohol/TAU group. Therefore, our results indicated, for the first time, that taurine administered acutely or chronically produces anxiolytic-like behavior dependent on the association with alcohol without altering locomotion, seen by the results on the first open field and on the light/dark test, respectively. Some studies have emphasized that

the increase of extracellular endogenous taurine in response to alcohol administration may reach about 150-200% of its basal concentration in several brain regions of rats (Ericson et al., 2011; Quertemont and Grant, 2004; Quertemont et al., 1999). Taurine was shown to produce a two-phase effect on zebrafish behavior when associated with alcohol, depending on its dose and alcohol exposure concentration (Rosemberg et al., 2012). In general, taurine prevented behavioral changes by low alcohol doses and potentiated the behavioral effects of high ethanol doses (Aragon et al., 1992). Thus, a higher concentration of taurine can be obtained in animals that consumed alcohol and were treated with taurine when compared to animals only treated with taurine. A synergistic effect between taurine and alcohol may have also occurred, potentiating its individual effects. This could be an explanation also to rearing behavior be reduced in Alcohol/TAU group at the first exposure to the open field, since the potentiated effects of alcohol could generate coordination impairment and motor balance.

On the second open field test, all animals reduced ambulation compared with that on the first test. This represents an indicative of habituation by repeated exposure to the same test. Therefore, measures of anxiety were impaired on this second exposure, resulting on the disappearance of most of significances seen on the first open field test. However, the only parameter that remained significantly increased from the first to the second exposure was the increase promoted by taurine in the latency for grooming in both treatment groups, as well as in the second test, taurine also significantly reduced the frequency of grooming in the same groups of animals. In fact, grooming behavior does not seem to produce habituation, since it has been shown to occur consistently even in up to seven repeated open field tests, and it may be considered an index of behavioral adaptation to a stressful situation, such as a new environment (Barros et al., 1994). Studies suggest that grooming behavior is regulated by GABAergic system, as

GABA agonists appear to reduce grooming, whereas this can be reverted by the association with a GABA antagonist (Barros et al., 1994; Kalueff and Tuohimaa, 2005). Thus, the reduction of grooming may be a manifestation of anxiolytic-like effect, related to the activation GABAergic system. Thus, it could be suggested that taurine acts as a GABA agonist in controls animals reducing grooming and ambulation, and in alcohol animals its effect is synergistic with alcohol, exacerbating the anxiolytic-like behavior.

Furthermore, in this study we did not observe locomotor changes or anxiolytic-like effect in animals of Alcohol/SAL group as could be expected. As in this experiment we have the 24 h measure of alcohol consumption and we have not the rats blood alcohol concentration, it may be suggest that these animals may were not under acute effect of alcohol on the moment of behavioral tests. Or it can also be suggested that these animals may has developed tolerance to the alcohol anxiolytic effects, related to neuroplasticity caused by chronic consumption of high amounts of alcohol and possibly down-regulation of $GABA_A$ receptors (Roberto and Varodayan, 2017). In agreement with this, it has been demonstrated that rodents that exerts a maintained high alcohol preference show decreased expression of some GABA transporters in the amygdala, increased GABA mediated inhibition and down-regulation of several $GABA_A$ receptor subunit transcripts in this brain area, accompanied by higher anxiety-like behavior with no effect in locomotion (Augier et al., 2018). However, it has been shown that oral taurine supplementation increased the expression of glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, and increased levels of GABA in the brain of mice (El Idrissi and Trenkner, 2004), although in the present study we did not also observe anxiolysis in Control/TAU animals. Thereby it can be suggested that taurine treatment in alcoholic group of animals could be restoring the functionality of these GABAergic receptors, possibly by compensatory mechanisms in response to the

alterations in this inhibitory system. Thus, the association between alcohol and taurine seemed to be determinant to the appearance of anxiolytic-like effect, possibly due to synergism that potentiates the GABAergic system activation.

5. CONCLUSION

Chronic taurine treatment increases the voluntary alcohol consumption and preference in rats, probably due to synergistic effect with alcohol that facilitates the activation of dopaminergic reward pathway. Taurine promotes an anxiolytic-like behavior only in alcohol treated rats, possibly emphasizing its synergistic effect, which occurs also on the GABAergic system. Although taurine did not show an anti-adictive effect, decreasing voluntary consumption in alcohol rats, we cannot discard that taurine, as acamprosate, shows therapeutic effects only in abstinent rats.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Legend of Figures

Figure 1: Alcohol preference (%). Determined by the ratio between the total amount of alcohol consumed and the total of both liquids consumed (alcohol + saccharin bottles) × 100. Considering the daily alcohol preferences from the beggining of taurine treatment. *P < 0.005, representing the significantly higher preference for alcohol by Alcohol/TAU group compared to Alcohol/SAL group.

Figure 2: Daily alcohol consumption (g/kg). The point zero corresponds to the basal intake, obtained by the average daily alcohol intake of the days before the intervention. *P < 0.001, representing the significantly higher alcohol consumption by Alcohol/TAU group in related to Control/SAL group that occurs since the day 6 of taurine administrations. #P < 0.049 represents the significant increase on the global alcohol consumption of taurine treated rats in relation to controls.

Figure 3: First open field test after acute taurine administration. (A) Total ambulation; (B) Peripheral crossings; (C) Central crossings; (D) Latency time for center output; (E) Latency time for the first grooming, *P = 0.040, TAU groups significantly different from SAL groups.; (F) Frequency of grooming; (G) Frequency of rearing; (H) Number of fecal boli.

Figure 4: Second open field after chronic taurine treatment. (A) Total ambulation; (B) Peripheral crossings; (C) Central crossings; (D) Latency time for center output; (E) Latency time for the first grooming, *P = 0.024, TAU groups significantly different from SAL groups.; (F) Frequency of grooming, *P= 0.048, TAU groups significantly different from SAL groups.; (G) Frequency of rearing; (H) Number of fecal boli.

Figure 5: Light/dark test after chronic taurine treatment. (A) Time spent in the light compartment; (B) Number of transitions between light and dark compartments; (C) Latency time to enter for the first time in the dark compartment; (D) Frequency of risk assessment behavior.

Figure 1

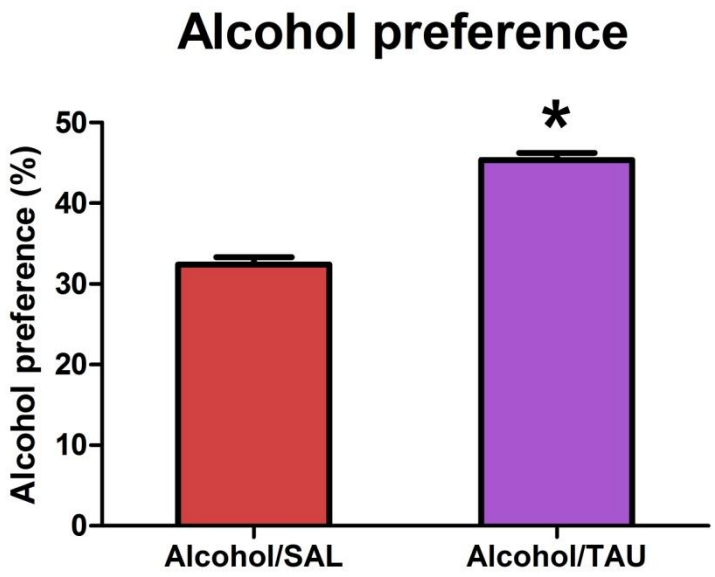


Figure 2

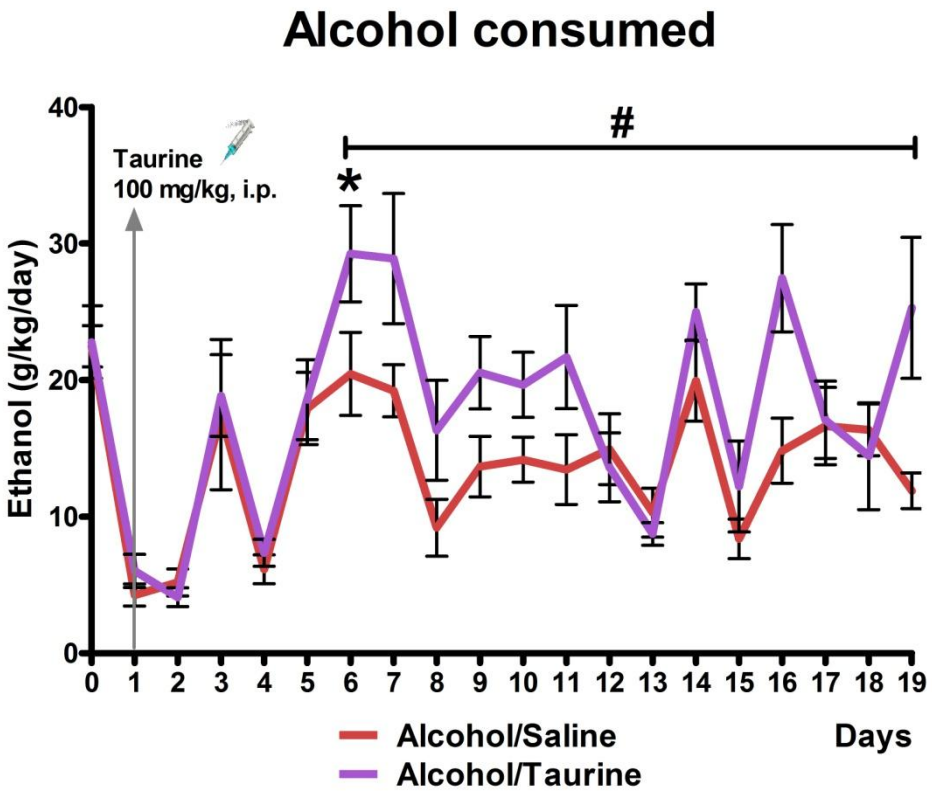


Figure 3

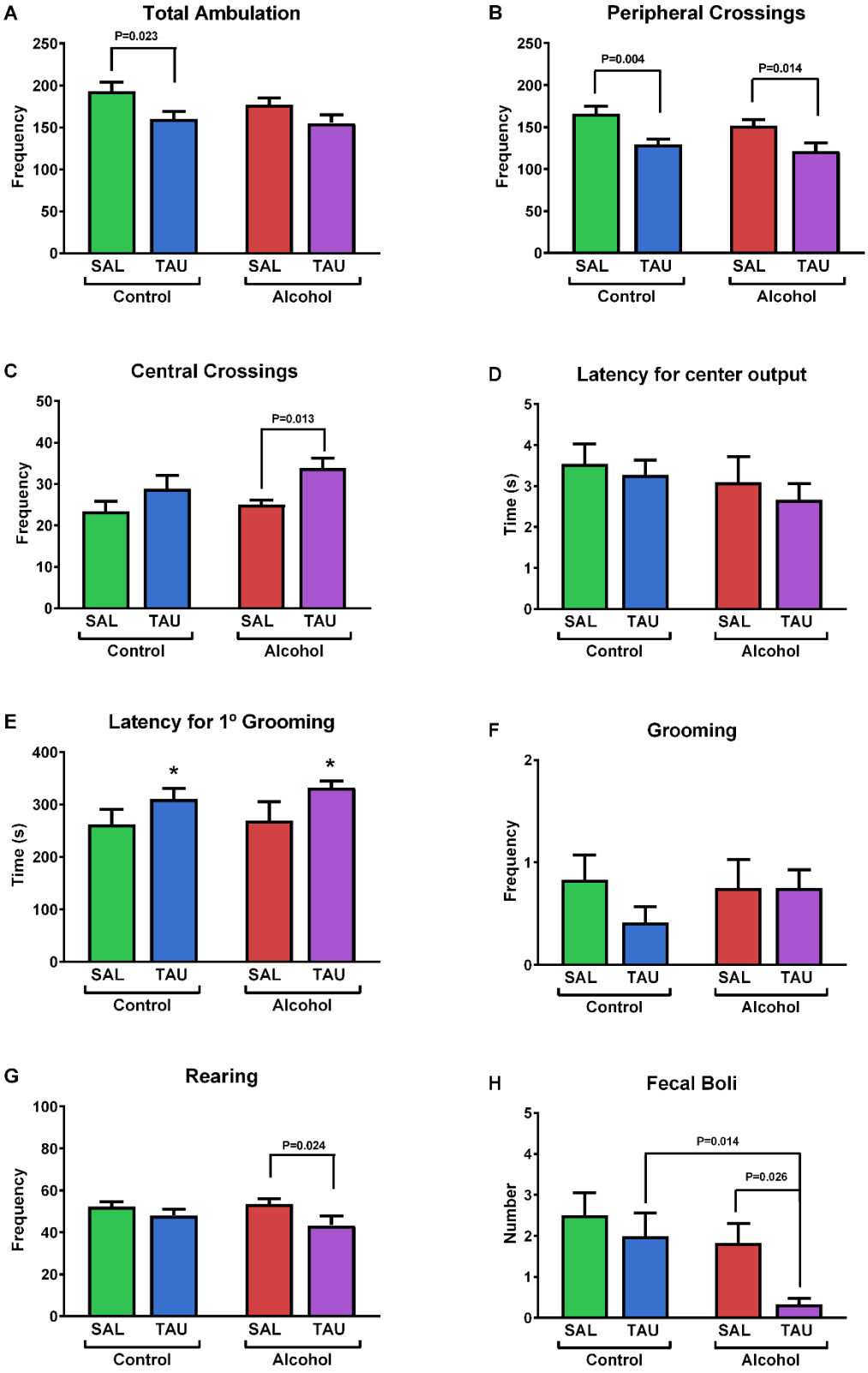

A
Total Ambulation
P=0.023
Frequency
250
200
150
100
50
0
SAL
TAU
SAL
TAU
Control
Alcohol
B
Peripheral Crossings
P=0.004
P=0.014
Frequency
250
200
150
100
50
0
SAL
TAU
SAL
TAU
Control
Alcohol
C
Central Crossings
P=0.013
Frequency
50
40
30
20
10
0
SAL
TAU
SAL
TAU
Control
Alcohol
D
Latency for center output
Time (s)
5
4
3
2
1
0
SAL
TAU
SAL
TAU
Control
Alcohol
E
Latency for 1º Grooming
*
*
Time (s)
400
300
200
100
0
SAL
TAU
SAL
TAU
Control
Alcohol
F
Grooming
Frequency
2
1
0
SAL
TAU
SAL
TAU
Control
Alcohol
G
Rearing
P=0.024
Frequency
100
80
60
40
20
0
SAL
TAU
SAL
TAU
Control
Alcohol
H
Fecal Boli
P=0.014
P=0.026
Number
5
4
3
2
1
0
SAL
TAU
SAL
TAU
Control
Alcohol

Figure 4

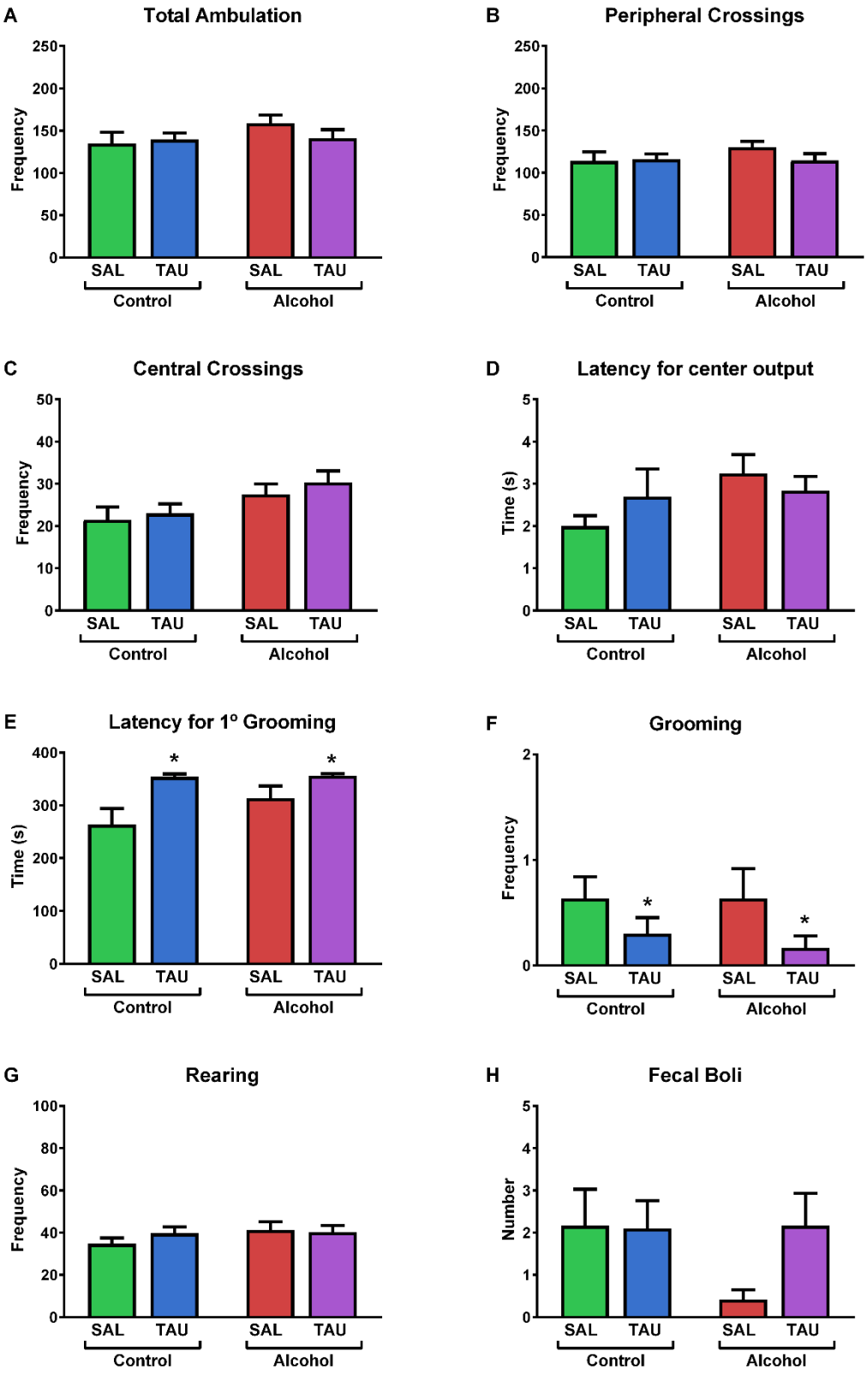
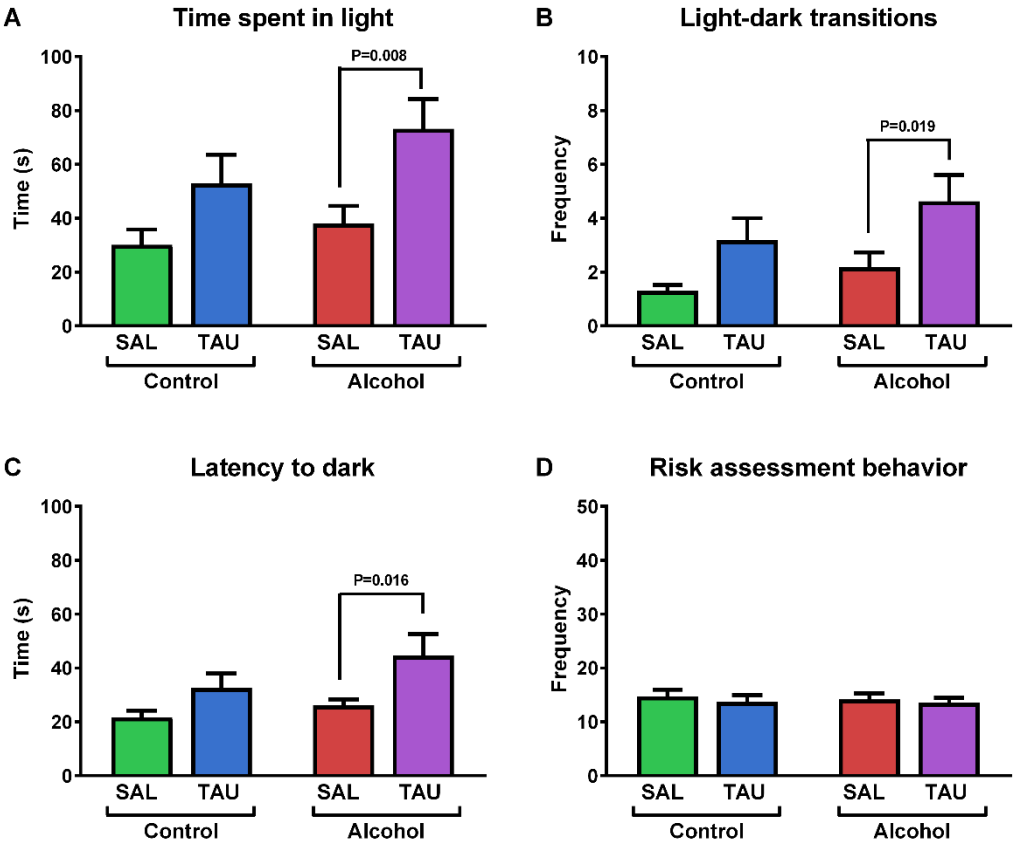

A
Total Ambulation
Frequency
250
200
150
100
50
0
SAL
TAU
SAL
TAU
Control
Alcohol
B
Peripheral Crossings
Frequency
250
200
150
100
50
0
SAL
TAU
SAL
TAU
Control
Alcohol
C
Central Crossings
Frequency
50
40
30
20
10
0
SAL
TAU
SAL
TAU
Control
Alcohol
D
Latency for center output
Time (s)
5
4
3
2
1
0
SAL
TAU
SAL
TAU
Control
Alcohol
E
Latency for 1° Grooming
Time (s)
400
300
200
100
0
*
*
SAL
TAU
SAL
TAU
Control
Alcohol
F
Grooming
Frequency
2
1
0
*
*
SAL
TAU
SAL
TAU
Control
Alcohol
G
Rearing
Frequency
100
80
60
40
20
0
SAL
TAU
SAL
TAU
Control
Alcohol
H
Fecal Boli
Number
5
4
3
2
1
0
SAL
TAU
SAL
TAU
Control
Alcohol

Figure 5


A
Time spent in light
Time (s)
100
80
60
40
20
0
P=0.008
SAL
TAU
SAL
TAU
Control
Alcohol
B
Light-dark transitions
Frequency
10
8
6
4
2
0
P=0.019
SAL
TAU
SAL
TAU
Control
Alcohol
C
Latency to dark
Time (s)
100
80
60
40
20
0
P=0.016
SAL
TAU
SAL
TAU
Control
Alcohol
D
Risk assessment behavior
Frequency
50
40
30
20
10
0
SAL
TAU
SAL
TAU
Control
Alcohol

5. CONCLUSÕES

Tratamento com taurina aumenta o consumo voluntário e preferência por álcool em ratos, possivelmente devido à restauração de neuro-adaptações do sistema dopaminérgico induzidas pelo uso crônico de álcool ou devido a um efeito sinérgico com o álcool neste sistema, facilitando a ativação de vias neurais de recompensa dopaminérgica. No entanto, não descartamos que taurina possa estar participando também de adaptações fisiológicas produzidas pelo consumo crônico de altas quantidades de álcool, reduzindo os seus efeitos indesejados e assim aumentando o seu consumo.

Administração aguda ou crônica de taurina produz efeito tipo-ansiolítico em ratos dependente da associação com o álcool, sem alterar a locomoção, possivelmente enfatizando o seu efeito sinérgico com o álcool que ocorre também na ativação do sistema GABAérgico. Taurina poderia também estar restaurando a funcionalidade de receptores GABAérgicos alterados pelo uso crônico de álcool, possivelmente por mecanismos compensatórios.

Embora neste estudo a taurina tenha aumentado o consumo de álcool em ratos, não descartamos que esta poderia, assim como o seu derivado sintético acamprosato, exercer efeitos terapêuticos apenas após abstinência de álcool. Desse modo, dando continuidade a este trabalho, temos como perspectiva a avaliação do efeito da taurina sobre o consumo voluntário de álcool em ratos após um período de abstinência.

Estudos adicionais serão realizados a fim de elucidar os mecanismos pelos quais a taurina promove estes efeitos em associação ao uso continuado de álcool. Estes mecanismos serão investigados pela análise de parâmetros neuroquímicos intracelulares e extracelulares, como a marcação de receptores e dosagens de biomarcadores

envolvidos. A possibilidade de influência da taurina sobre o metabolismo do álcool também será explorada pela avaliação de variações bioquímicas centrais e periféricas.

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enhancement of environmental habituation in mice. Pharmacol. Biochem. Behav. *101*, 132–137.

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ANEXO

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 32850
Título: EFEITO DA TAURINA SOBRE O CONSUMO VOLUNTARIO DE ALCOOL E SUA RELACAO COM OS SISTEMAS GABAERGICO E GLUTAMATERGICO DE RATOS

Vigência: 01/04/2017 à 30/03/2019

Pesquisadores:

Equipe UFRGS:

ROSANE GOMEZ - coordenador desde 01/04/2017
RIANNE REMUS PULCINELLI - Aluno de Mestrado desde 01/04/2017
Solange Bandiera - Aluno de Doutorado desde 01/04/2017
NATÁLIA AZUAGA NIETIEDT - Aluno de Especialização desde 01/04/2017

Equipe Externa:

Fernanda Urruth Fontella - pesquisador desde 01/04/2017

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 05/06/2017 - SALA 330 DO ANEXO I DO PRÉDIO DA REITORIA - CAMPUS CENTRO - UFRGS-PAULO DA GAMA, 110 BAIRRO FARROUPILHA - em seus aspectos éticos e metodológicos, para a utilização de 48 ratos machos Wistar de 270 g, provenientes do CREAL/UFRGS; de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Sexta-Feira, 16 de Junho de 2017

MARCELO MELLER ALIEVI
Coordenador da comissão de ética