The combination of Galanin (1-15) and Escitalopram decrease the alcohol self-administration in rats through the functional network ventral tegmental area-dorsal raphe

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INTRODUCTION

Alcohol Use Disorder (AUD) is a highly prevalent, and **most AUD patients suffer comorbidity with depression**. Selective 5-HT reuptake inhibitors (SSRIs) can reduce rodent alcohol drinking but exert modest clinical efficacy in alcoholic individuals. Recently, we have described that the neuropeptide N-terminal of Galanin (1-15) **[GAL(1-15)] induces a reduction in voluntary alcohol consumption in rats, with involvement of the dopaminergic mesolimbic system**, moreover, **GAL(1-15) enhance the antidepressant effects induced by Escitalopram (ESC)** in depression-related behavioral tests.

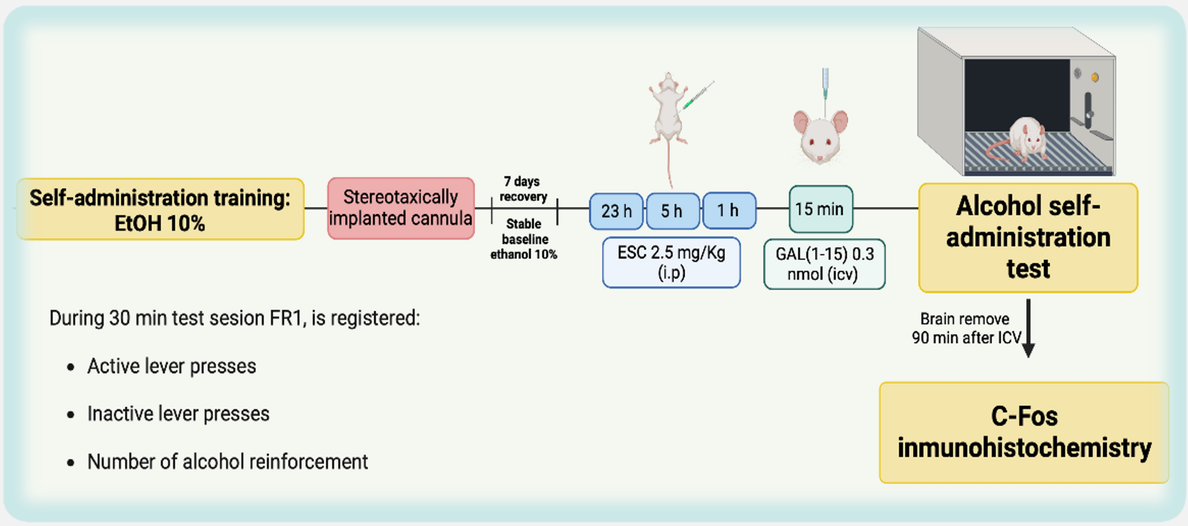
OBJECTIVE: To investigate the effect of GAL(1-15) on ESC-mediated effect in depression-alcoholism comorbidity, we used the **alcohol self-administration test.** In addition, to study the circuits involved, **we analyzed the immunohistochemistry of C-Fos in several nuclei implicated in depression and AUD:** dorsal raphe (DR), rostromedial tegmental nucleus (RMTg), lateral habenula (LHb), medial habenula (mHb), ventral tegmental area (VTA), nucleus accumbens (NAc) and prefrontal cortex (PFC) and **we assessed the brain circuits using principal component analysis** (PCA) **to understand brain functional organization.**

MATERIAL AND METHODS

- Male Sprague-Dawley rats were stereotixically implanted with a unilateral chronic cannula into the lateral cerebral ventricle according to the atlas of Paxinos and Watson.

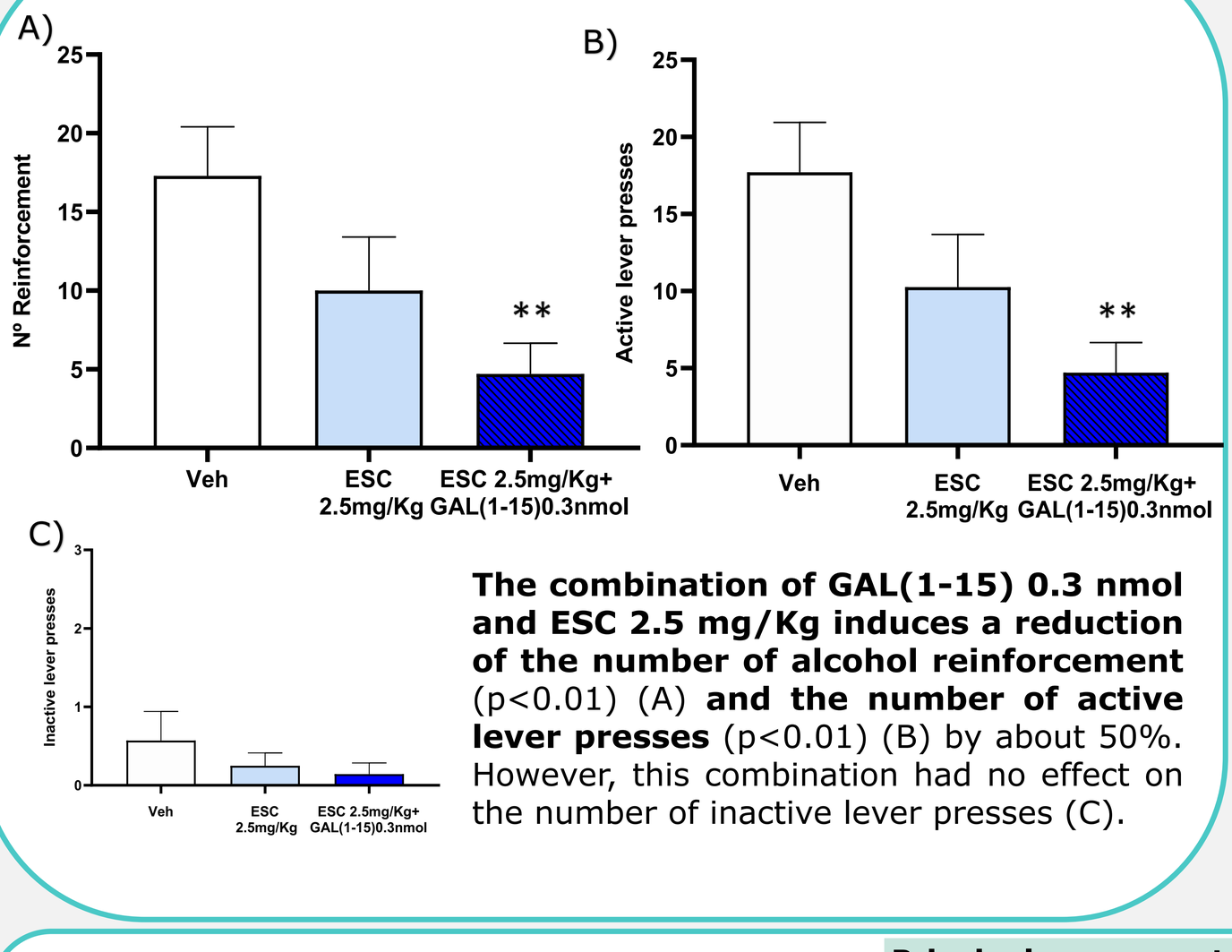
1. **Alcohol self-administration: rats were trained to self-administer ethanol 10%** on a fixed ratio 1 schedule of reinforcement. The active lever was paired with the delivery of ethanol as a reward, whereas the inactive lever was paired with no reward. **Group of rats received three intraperitoneal injections of ESC (2.5 mg/Kg) 23, 5 and 1h before the test and one icv injection of GAL (1-15) (0.3 nmol) 15 min before the test**. During the 30 min test sessions, the responses on the active lever, inactive lever and number or alcohol reinforcement were recorder.
2. The brains were removed 90 minutes after the icv injection to **analyzed the immunoreactivity of C-Fos in DR, RMTg, LHB, mHB, VTA, NAc and PFC.**
3. A PCA with varimax rotation was also performed to extract the independent factors from the C-Fos IR data.

- One-way ANOVA followed by Fisher´s least significant difference test was used.



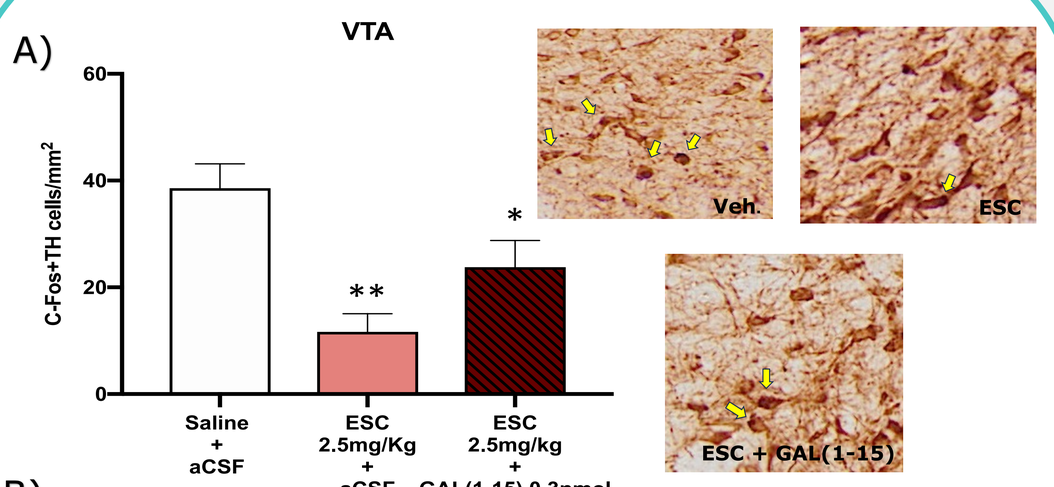
RESULTS

Alcohol self-administration test



The combination of GAL(1-15) 0.3 nmol and ESC 2.5 mg/Kg induces a reduction of the number of alcohol reinforcement ($p<0.01$) (A) **and the number of active lever presses** ($p<0.01$) (B) by about 50%. However, this combination had no effect on the number of inactive lever presses (C).

C-Fos Immunohistochemistry

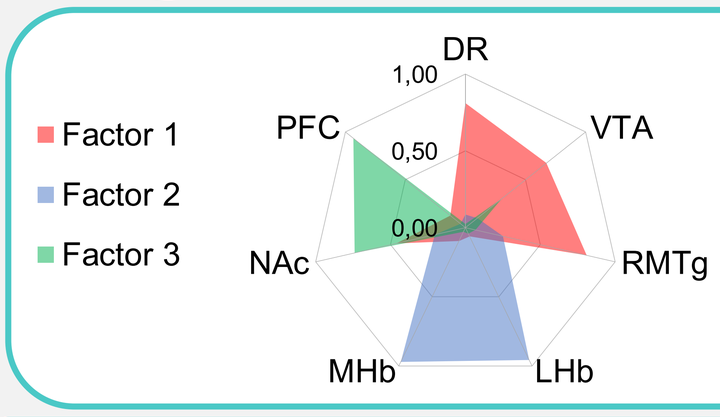


B)

Treatment	RMTg	LHb	MHb	NAc	PFC	RD
	C-Fos cells/mm²					C-Fos+5HT cells/mm²
Saline + aCSF	60.8 ± 22.2	176.0 ± 57.9	218.4 ± 75.1	33.6 ± 8.1	259.2 ± 44.2	37.8 ± 10.3
ESC 2.5 mg/Kg + aCSF	21.0 ± 6.5	180.3 ± 74.3	205.5 ± 82.7	20.4 ± 5.4	210.3 ± 34.3	42.8 ± 6.7
ESC 2.5 mg/Kg +GAL(1-15) 0.3 nmol	51.6 ± 10.2	371.7 ± 113.1	406.6 ± 94.9	40.9 ± 13.2	327.9 ± 34.9	42.7 ± 8.5

The number of C-Fos IR TH cell bodies **after combination of GAL(1-15) 0.3 nmol + ESC 2.5 mg/Kg** ($p<0.05$) **and ESC** ($p<0.01$) **was significantly decreased** in comparation with C-Fos IR TH cell bodies in the control group (A). No significative effects were observed in the rest of the nuclei (B).

Principal component analyses



Brain nuclei	Factor 1	Factor 2	Factor 3
Rafe	**-.811**	.085	.023
VTA	**.674**	-.100	.303
RMTg	**.810**	.250	-.068
LHb	-.064	**.957**	.044
MHb	.097	**.970**	.031
NAc	.461	.203	**.741**
PFC	-.126	-.041	**.933**
Eigenvalue	2.35	1.82	1.33
Variance explained (%)	33.60	26.10	19.02

The PCA revealed **three independent factor representing the functional brain networks** that explainded around 80% of the total variance. **The first encompassed DR, VTA and RMTg** (33.60% of variance explained). The second and the third factor were composed of LHb and mHB and Nac and PFC.

CONCLUSIONS

Our results indicate:

- A potent effect of the **combination GAL(1-15) with ESC in reducing the reward-seeking motivated by alcohol.**
- A **functional network**, consisting mainly of **DR, VTA and RMTg,** is involved in **GAL(1-15)+ESC alcohol self-administration effects.**
- It opens up the possibility to use **GAL(1-15) in combination with ESC as a novel strategy in AUD comorbidity with depression.**

Supported by PID2020-114392RB-100, PDC2021-121566-100, P20-00026-R, PI-0083-2019

The Combination of Galanin (1-15) and Escitalopram in Rats Suggests a New Strategy for Alcohol Use Disorder Comorbidity with Depression. Biomedicines. 2022 Feb 9;10(2):412. doi: 10.3390/biomedicines10020412.