Translational Psychiatry

https://doi.org/10.1038/s41398-026-03838-5

Article in Press

Effects of chronic ethanol consumption on brain GLP-1R gene expression in mice and humans

Received: 11 July 2025

Revised: 12 December 2025

Accepted: 20 January 2026

Cite this article as: Torregrosa, A.B., García-Gutiérrez, M.S., Ortuño-Miquel, S. *et al.* Effects of chronic ethanol consumption on brain GLP-1R gene expression in mice and humans. *Transl Psychiatry* (2026). https://doi.org/10.1038/s41398-026-03838-5

Abraham B. Torregrosa, María S. García-Gutiérrez, Samanta Ortuño-Miquel & Jorge Manzanares

Original article

Effects of chronic ethanol consumption on brain GLP-1R gene expression in mice and humans

Abraham B. Torregrosa[a,b,c], María S. García-Gutiérrez[a,b,c], Samanta Ortuño-Miquel[c] and Jorge Manzanares[a,b,c*]

[a] Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, Avda de Ramón y Cajal s/n, San Juan de Alicante, 03550 Alicante, Spain.

[b] Red de Investigación en Atención Primaria de Adicciones, Instituto de Salud Carlos III, MICINN and FEDER, Madrid, Spain.

[c] Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, Spain.

Running title:

GLP-1R: new potential biomarker for AUD

*Corresponding author. Phone: +34-96-591-9252

E-mail address: jmanzanares@umh.es

Abstract

The glucagon-like peptide-1 receptor (GLP-1R) has emerged as a promising therapeutic option for alcohol use disorder (AUD), yet the underlying mechanisms and neurocircuitry involved remain unclear. This study aimed to analyze GLP-1R gene expression changes in brain regions associated with alcohol's effects, including the prefrontal cortex (PFC), nucleus accumbens (NAc), and hippocampus (HIP), in mice following 42 days of voluntary ethanol consumption (VEC; 10% v/v) and postmortem samples from 18 patients with AUD. Additionally, we examined the expression of OPRM1 (mu-opioid receptor) and BDNF (brain-derived neurotrophic factor), key targets related to alcohol intake and reward, in the NAc and HIP, respectively.

GLP-1R gene expression was significantly reduced in all brain regions of ethanol-exposed mice and AUD patients. These reductions paralleled decreased OPRM1 and BDNF expression in the NAc and HIP, respectively. Pearson and Spearman correlation analyses revealed no significant associations between gene expression and age, RIN, pH, postmortem interval (PMI), body mass index (BMI), smoking status, age of onset of alcohol use, or years of drinking.

In summary, chronic alcohol consumption in humans or mice was associated with decreased GLP-1R gene expression in brain regions involved in the reinforcing effects of ethanol. These findings open new avenues for further research into how this emerging receptor could serve as a potential biomarker and therapeutic target in AUD.

Keywords: postmortem study, mice, AUD, voluntary ethanol consumption, gene expression, GLP-1R.

1. Introduction

Alcohol use disorder (AUD) represents a complex psychiatric condition associated with elevated rates of morbidity and mortality on a global scale[1]. The World Health Organization reports that around 400 million individuals worldwide are affected by AUD[2]. Alcohol consumption accounts for about 3 million deaths each year and constitutes a leading cause of premature mortality among individuals aged 15 to 49. Despite this, treatment options remain limited, with only three medications approved by the Food and Drug Administration—namely, acamprosate, disulfiram, and naltrexone—and exhibit limited efficacy[3]. Therefore, there is an urgent need to find new pharmacological treatments that improve the clinical outcome of patients with AUD.

In this regard, preclinical and clinical data indicate that the glucagon-like peptide-1 (GLP-1) system, acting through its receptor (GLP-1R) as part of a gut–brain signaling pathway, plays a critical role in the neurobiology of addictive behaviors [4,5]. The GLP-1R is a class B G protein-coupled receptor that activates cAMP/PKA signaling as well as PI3K/Akt, MAPK/ERK and β-arrestin pathways [6-9]. Notably, it is widely distributed in the brain, including the nucleus accumbens (NAc), hippocampus (HIP), cortical regions, and ventral tegmental area, and is expressed across glutamatergic, GABAergic, and subsets of dopaminergic neurons [10-13]. This signaling and widespread distribution point out its potential to modulate alcohol reinforcing properties.

Specifically, recent studies have shown that GLP-1 receptor (GLP-1R) agonists reduce ethanol intake, motivation to consume ethanol, relapse drinking, and symptoms of abstinence in rodent models[14-17]. Notably, the reduction in alcohol intake caused by GLP-1R agonists is not entirely specific, as earlier studies have also observed simultaneous decreases in food and water intake [18,19]. However, recent studies show a reduction in ethanol intake without changing food or fluid consumption [20-22]. Early clinical studies have shown that GLP-1R agonists reduce alcohol intake in patients with AUD and comorbidities of obesity and type 2 diabetes[23-25]. Moreover, genetic variations in genes encoding for the GLP-1R have been associated with AUD and heavy

drinking[26,27]. Taken together, the evidence provided to date raises important questions about the therapeutic use of GLP-1R agonists in individuals with AUD who are not overweight or diabetic, highlighting the need to distinguish between direct effects on alcohol-related neurocircuitry and broader influences on energy balance and eating behaviors.

To attain a more comprehensive understanding of the interaction between GLP-1 and alcohol, further investigation is urged to examine the potential impact of alcohol on the endogenous GLP-1 system. In rodents, alcohol exposure altered GLP-1 signaling in brain reward regions, including the NAc[28] and the frontal lobe[29]. In humans, indirect evidence provided by neuroimaging and biomarker studies showed that chronic alcohol use alters brain circuits involving GLP-1 pathways[26,30]. However, postmortem brain studies specifically measuring GLP-1R expression after chronic alcohol use are scarce[31].

In this study, we performed a comprehensive analysis of the impact of chronic alcohol consumption on GLP-1R gene expression within PFC, NAc, and HIP in both rodent models and patients diagnosed with AUD. Furthermore, we conducted additional examinations of two pivotal markers associated with alcohol dependence: the mu opioid receptor (OPRM1) in the NAc and brain-derived neurotrophic factor (BDNF) in the HIP. OPRM1, which encodes the mu opioid receptor, has been consistently associated with alcohol reward, craving, and treatment response, making it one of the most studied candidate genes in the context of AUD [32-34]. Similarly, BDNF plays a crucial role in synaptic plasticity and neuroadaptation processes underlying alcohol dependence and relapse, and altered BDNF signaling has been reported in both clinical and preclinical models of AUD [35-37]. The HIP was prioritized for BDNF, given its critical role in learning, memory, and neuroplasticity —processes directly affected by chronic alcohol exposure [38,39]. On the other hand, OPRM1 expression was examined in the NAc due to its well-established involvement in reward processing and alcohol reinforcement, where mu-opioid receptor signaling is particularly relevant [40,41]. While both genes are functionally relevant in additional brain regions,

the selection was guided by their strongest mechanistic and experimental links with the HIP and NAc, respectively.

2. Materials and methods

2.1 Animals

Male C57BL/6J mice from Charles River in Lille, France, aged six weeks and weighing 20- 25 grams, were housed in groups of five in enclosures (40 × 25 × 22 cm) under controlled conditions (temperature 21 ± 2°C, humidity 60 ± 10%, 12-hour light/dark cycle from 08:00 to 20:00). Behavioral tests started one week after acclimation. All procedures complied with Spanish Royal Decree 1201/2005, Law 32/2007, and EU Directive 2010/2010/E.

2.2. Voluntary Ethanol Consumption in a Two-Bottle Choice Paradigm (VEC)

This paradigm was carried out according to a protocol previously described[42,43]. All mice were housed individually in cages with two water bottles to facilitate acclimatization and reduce stress. After a week, the two-bottle choice paradigm began: one bottle contained water, and the other contained a solution of increasing ethanol concentrations (2%, 4%, 6%, 8%, 10%) or water (controls), administered every 3 days. Once the ethanol concentration reached 10% for three consecutive days, consumption and preference were measured over a period of six weeks. Solutions were prepared and changed daily between 18:00 and 19:00, with bottles being alternated to avoid bias. Daily intake was recorded, and ethanol intake was calculated in grams per kilogram body weight per day (g/kg/day). The ethanol preference ratio was calculated as ethanol intake divided by total intake.

2.3. Gene expression studies by real-time PCR (qPCR) in mice

Mice were sacrificed at the end of VEC. Briefly, brains were removed from the skull, frozen on dry ice, and stored at -80°C until the testing day. Brain sections measuring 500 µm were cut at various levels, including the regions of interest (PFC, NAc and HIP), according to the Paxinos and Franklin atlas[44]. The sections were then mounted on slides and stored at -80°C. A section from each level was dissected according to the method outlined by Palkovits [45]. Total RNA was isolated from brain punctures using Biozol® Total RNA Extraction Reagent (Bioflux, Inilab, Madrid, Spain) in the PFC, NAc and HIP regions. Following DNase digestion, reverse transcription was performed to generate complementary DNA (cDNA) according to the manufacturer's instructions (High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, Applied Biosystems, Madrid, Spain). Quantitative analyses of relative gene expression for GLP-1R (Mm00445292_m1), OPRM1(Mm01188089_m1), and BDNF (Mm00432069_m1) were conducted using the Taqman Gene Expression Assay (Applied Biosystems, Madrid, Spain), which uses a specific fluorescent dye for double-stranded DNA. These analyses were performed on the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Madrid, Spain). The reference gene employed was 18S rRNA (Mm03928990_g1), detected with Taqman ribosomal RNA control reagents. Briefly, data for each target gene were normalized to the endogenous reference gene, and changes in target gene abundance were determined using the $2^{-\Delta\Delta CT}$ method [46].

2.4. Human samples

Postmortem frozen brain samples obtained from patients with AUD (n=18) and their corresponding control subjects (C) (n=18) were kindly provided by the New South Wales Tissue Resource Centre (NSWBTRC) at the University of Sydney in Australia. This brain bank nation has a significant population of individuals experiencing alcohol dependence without the co-abuse of other substances. Thus, this population with AUD of Australia represents a unique resource for

researchers exploring the long-term effects of alcohol on the brain. The brain regions available were the prefrontal cortex (PFC) (Brodmann area 9), NAc, and HIP.

The patients met the criteria established in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) for AUD. Details on the methods used at the NSWBTRC to collect demographic, alcohol-related, and other behavioral data have been previously reported [47]. Mean age of beginning drinking is 19.9 ± 4.5 years, and years of consumption are 35.9± 8.9. Additional demographic and postmortem data for all subjects included in the present study are described in Table 1. None of the AUD cases were receiving treatment at the time of death.

Patients did not present any infectious disorders, including Hepatitis B and C, HIV and AIDS, or Creutzfeldt-Jakob disease.

All participants were Caucasian males and were matched as closely as possible regarding age (AUD: 55.8 ± 9; C: 56.3 ± 9.4), postmortem interval (AUD: 39.7 ± 16.9 h; C: 31.8 ± 13.2 h) and pH (AUD: 6.6 ±0.2, C: 6.7±0.2).

2.5. RNA integrity number (RIN) evaluation

RNA quality in human samples is crucial for reliable qPCR results. Total RNA was isolated from PFC, NAc, and HIP tissues using TRI Reagent (Applied Biosystems). RNA integrity was assessed with the 2100 Bioanalyzer, which detects 18S and 28S ribosomal bands. The ratio of 28S to 18S yields the RNA Integrity Number (RIN), which ranges from 1 to 10, with lower values indicating greater RNA degradation. Electropherograms and gel images of RNA from control and experimental subjects (Fig. 1) showed a mean RIN of about 7 (PFC C: 6.85±0.105, AUD: 6.905±0.125; NAc C: 6.876±0.079, AUD: 6.829±0.1050.127; HIP C: 6.867±0.009, AUD: 6.861±0.014), indicating good RNA quality for gene expression analysis.

2.6. Gene expression studies by real-time PCR (qPCR) in humans

Relative gene expression analyses of GLP-1R, OPRM1 and BDNF in the PFC, NAc, and HIP were conducted in A and C following the same methodology as previously described for mice using the TaqMan Gene Expression Assays (GLP-1R: Hs00157705_m1, OPRM1: Hs1053957_m1, BDNF: Hs02718934_s1). Two reference genes, cyclophilin (PPIA, Hs99999904_m1) and synaptophysin (SYP, Hs00300531_m1), were employed to ensure the validity and reproducibility of the results. For each sample, the results are presented as the average derived from each reference gene.

The number of samples analyzed in the human qPCR studies varies based on the number of samples available.

2.7. Statistical analysis

Normality of numeric variables was assessed using the Shapiro–Wilk test (given the sample size below 50 patients). For normally distributed variables, results are presented as mean ± standard deviation, and comparisons between groups were performed using the Student's t-test. For non-normally distributed variables, results are presented as median (25th–75th percentile), and comparisons were carried out using the Mann–Whitney U test. Categorical variables are expressed as absolute frequencies and percentages. A p-value < 0.05 was considered statistically significant.

Two-way ANOVA with repeated measures followed by Student-Newman-Keuls was used to analyze body weight in ethanol-exposed mice and their corresponding control group through the VEC.

2.7.1. Correlation analysis

For normally distributed numeric variables, correlations were assessed using Pearson's correlation coefficient, while Spearman's rank correlation coefficient was applied for non-normally distributed variables.

2.7.2. Linear Regression

To analyze gene expression, linear regression models were developed to analyze gene expression, incorporating globally significant variables identified through statistical significance ($p < 0.20$) and correlation analyses. Rather than performing separate regressions for each biomarker, all variables that showed significance in at least one marker were included together in a unified model. Three models were tested:

1. Model 1 included only the group variable.
2. Model 2 included age, group, clinical history, cause of death, smoking history and BMI.
3. Model 3 included the same variables as Model 2, with the addition of an age × group interaction term.

All statistical analyses were conducted using R (version 4.5.0), employing the following packages: *dplyr* (1.1.4), *readr* (2.1.5), *tidyr* (1.3.1), *gtsummary* (2.3.0), *ggplot2* (3.5.2), and *ggpubr* (0.6.1). SigmaPlot 11 software (Systat Software Inc., Chicago, IL, USA) was used to create figures. The code used in this study is available in the GitHub repository (https://github.com/Samantao93/Biomarkers_AUC).

3. Results

3.1. Voluntary Ethanol Consumption (VEC) of C57BL6J male mice

As illustrated in Figure 2A, animals subjected to VEC demonstrated ethanol consumption levels of approximately 5 g/kg per day, along with an ethanol consumption preference ranging from 50% to 60% (Figure 2B).

A two-way ANOVA with repeated measures with group as a between-subject factor and week as a within-subject factor revealed no significant main effect of group ($F_{(1, 18)}$ = 0.61, p = 0.446) and no significant group × week interaction ($F_{(5, 90)}$ = 0.98, p = 0.436), indicating that body weights did not differ between groups during VEC. As expected, there was a significant main effect of week ($F_{(5, 90)}$ = 337.49, p < 0.001), reflecting normal weight gain over time (Figure S1).

3.2. GLP-1R, OPRM1 and BDNF gene expression in the PFC, NAc and HIP of C57BL/6J male mice subjected to VEC

GLP-1R gene expression decreased in the PFC (Figure 3A; Student t-test: t= 2.351, 16df, p= 0.032) (n=8-10/group), NAc (Figure 3B; Student t-test: t= 2.219, 17df, p= 0.040) (n=9-10/group), and HIP (Figure 3C; Student t-test: t= 2.851, 15df, p=0.012) (n=7-10/group) of mice exposed to VEC in comparison to the control group.

Analysis of OPRM1 gene expression showed a significant reduction in the NAc (Figure 3D; Mann–Whitney U test; *U* = 89, *p* = 0.004) (n=10-11/group) of mice exposed to ethanol compared to the control group.

Besides, a significant reduction of BDNF was also found in the HIP (Figure 3D; Student t-test: t= 4.646, 15 df, p<0.001) (n=7-10/group) of mice exposed to ethanol compared to their control group.

Correlation analyses were conducted to examine the relationship between alcohol intake and the expression of GLP-1R, OPRM1 and BDNF across the different brain regions in the rodent model. No statistically significant correlations were observed. A moderate, non-significant correlation was found between alcohol intake and GLP-1R in the HIP (Spearman's ρ = 0.59, p = 0.07). Correlation coefficients for all markers are presented in Table S1 and the overall pattern is illustrated in Figure S2 (correlation heatmap).

3.3. GLP-1R, OPRM1 and BDNF gene expression in the PFC, NAc and HIP of humans with AUD

Notably, GLP-1R gene expression was reduced in all the brain areas analyzed, the PFC (Figure 4A; Mann–Whitney U test; $U = 235$, $p < 0.001$) (n=14-18/group), NAc (Figure 4B; Student t-test: t= 3.208, 29df, p=0.003) (n=15-16/group) and HIP (Figure 4C; Student t-test: t= 4.998, 30df, p<0.001) (n=14-18/group), of AUD in comparison to C.

OPRM1 gene expression revealed a significant reduction in the NAc (Figure 4D; Student t-test: t= 2.801, 30df, p=0.009) (n=15-17/group) in AUD compared to C. Similarly, the analysis of BDNF gene expression revealed a significant reduction in the HIP (Figure 4E; Student t-test: t= 2.895, 30df, p=0.007) (n=14-18/group) in AUD compared to C.

Pearson's and Spearman product–moment correlation analyses revealed no significant associations between GLP-1R, OPRM1, and BDNF gene expression and age, postmortem interval (PMI), pH, RNA integrity number (RIN). Only a small, significant correlation was observed between GLP-1R in the PFC and PMI (r=0.71, p<0.01).

For BMI, Pearson's correlations were applied for all genes except GLP-1R in the PFC, where Spearman's method was used due to non-normal distribution. Moreover, Spearman's correlation analyses showed no significant associations between gene expression levels and either age of onset or years of alcohol consumption for GLP-1R, OPRM1, and BDNF, in cases A (Table 2).

Pairwise correlation analyses between GLP-1R, OPRM1, and BDNF gene expression within each brain region and in each group revealed no statistically significant correlations in the AUD group (Figure S3, panel A). Interestingly, in the C group, significant correlations were found between GLP-1R expression in the PFC and BDNF expression in the HIP ($r = 0.60$, $p < 0.05$), and between GLP-1R expression in the NAc and BDNF expression in the HIP ($r = –0.58$, $p < 0.05$) (Figure S3, panel B). Other correlations were weaker and not statistically significant.

Linear regression analyses were performed for each brain region. Adding group and significant descriptive variables increased the explained variance in several models. Inclusion of the age ×

group interaction term did not materially improve model fit, except for GLP-1R expression in the NAc, where the explained variance showed a modest increase to 36%, which remains relatively low, accounting for less than half of the total variance. Full model results are available in Table S2.

4. Discussion

This study further supports the strong link between alcohol exposure and the GLP-1R. The following evidence supports this finding: 1) chronic ethanol exposure reduces GLP-1R gene expression in the PFC, NAc, and HIP, brain areas closely associated with alcohol's reinforcing effects, in mice. 2) Interestingly, human postmortem studies revealed a significant reduction of the GLP-1R in these brain areas in individuals with AUD. 3) In both samples, GLP-1R alterations align with the changes found in OPRM-1 and BDNF in NAc and HIP, respectively.

The GLP-1R is widely expressed across the brain, including reward processing areas such as the VTA and NAc[10,48-50]. The cumulative evidence gathered in recent years indicates that the GLP-1R plays a significant role in modulating dopaminergic responses associated with alcohol within these brain regions[28,51,52]. This has led to the exploration of the potential efficacy of GLP-1R agonists in reducing alcohol consumption, with promising results in animal models[14,16,53-56] and human clinical studies [23-25].

The results revealed that the expression of GLP-1R was significantly decreased in all the brain regions analyzed (NAc, HIP and PFC) in male mice exposed to chronic ethanol consumption for 45 days, with a mean ethanol intake of 5 g/Kg body weight per day. Few previous studies have examined the impact of ethanol on GLP-1R, finding differences depending on the brain region analyzed, the type of alcohol exposure (acute vs. chronic), and the sex of the rodent. In these studies, no alterations in GLP-1R expression were detected in the PFC, VTA, amygdala, HIP, or striatum after ethanol exposure [28]. An increase of GLP-1R was found in the NAc and lateral septum of high alcohol-consuming rats in comparison to low alcohol-consuming rats [21,28].

Discrepancies between our results and those previously published may be related to the species used (mice vs. rats), the chronic ethanol consumption paradigm (two-bottle choice vs. intermittent access), or the duration of ethanol intake (45 days vs. 12 weeks). Our findings, therefore, expand upon prior work by examining multiple brain regions in mice under VEC, providing additional evidence for GLP-1R alterations associated with long-term ethanol exposure.

For instance, our study demonstrated a significant reduction in GLP-1R gene expression across corticolimbic brain regions in individuals with AUD. Specifically, we observed a downregulation of GLP-1R in the HIP, NAc, and PFC of patients with a long-term history of alcohol use. These results indicate similar changes in both types of samples (mice and humans) across all the analyzed areas.

Only one previous study examined changes in human postmortem brain tissues of male individuals diagnosed with severe AUD according to DSM-5, who also smoked[31]. In this study, an increase in GLP-1R was found in the HIP of individuals with AUD, with no differences in the PFC or NAc. The discrepancy in the changes of GLP-1R gene expression in the brain between both studies may be related to differences in alcohol patterns and consumption, the years of alcohol consumption, and/or the pharmacological treatment, among other potential factors.

Our findings support and expand upon previous preclinical evidence indicating that GLP-1R signaling influences alcohol-related behaviors. Animal studies have demonstrated that central activation of GLP-1R decreases alcohol intake and alcohol-seeking behaviors by lowering dopamine release in the NAc [15,53]. In this context, the reduced GLP-1R expression observed here may reflect a disturbance in endogenous inhibitory mechanisms regulating alcohol reward, which could potentially contribute to compulsive alcohol use. However, as we did not assess GLP-1R coupling or cell-type-specific expression, this interpretation should be considered hypothesis-generating, and future studies using functional and cellular approaches will be required to clarify the underlying mechanisms.

In the HIP and PFC, GLP-1R signaling contributes to neuroprotection and executive function [57-61]. The downregulation of GLP-1R gene expression in these brain regions may, at least in part, underlie alcohol-related cognitive deficits and impairments in decision-making and inhibitory control. Some human postmortem studies suggested a compensatory increase in brain GLP-1R expression[31], however, our results may reflect later-stage neuroadaptive or degenerative changes.

Interestingly, this study revealed that changes in GLP-1R are consistent with those found in two key neurobiological markers of alcohol addiction, the OPRM-1 and BDNF in the NAc and HIP. The mu opioid receptor (encoded by OPRM1) plays a central role in alcohol reward by modulating the hedonic and motivational effects of alcohol through the mesocorticolimbic circuit [62-64]. In our study, we observed reduced OPRM1 in the NAc in mice subjected to chronic ethanol exposure and in patients with AUD. Previous studies on this topic, however, reported heterogeneous findings: while several studies have described decreased OPRM1 in the NAc of mice exposed to ethanol [65-69] and in patients with AUD [70], others have reported increased expression or have focused on functional alterations (e.g., receptor coupling, signaling) rather than the gene expression per se [67,68]. This variability likely reflects the complex regulation of the mu opioid receptor, involving transcriptional, translational, and functional mechanisms. The reduced OPRM1 expression observed in our samples may reflect either receptor downregulation following chronic stimulation or neuroadaptive loss of opioid tone. Notably, lower OPRM1 expression has been associated with blunted reward sensitivity and an altered response to naltrexone treatment[71].

Interestingly, the analysis of BDNF gene expression, closely related to cognitive processes affected by alcohol consumption [72-76], revealed a significant reduction in mice and patients with AUD. These findings are consistent with prior studies showing that chronic alcohol exposure suppresses BDNF signaling, contributing to cognitive impairments and increased vulnerability to consume alcohol [77-80]. Despite further research is needed, the concurrent downregulation of both

BDNF and GLP-1R in the HIP may represent a convergent mechanism leading to hippocampal dysfunction in AUD.

Correlation analysis of gene-gene interactions within each brain area and across groups revealed distinct patterns between controls and AUD. In the control group, a positive correlation between GLP-1R expression in the PFC and BDNF expression in the HIP was observed, which may reflect a physiological interaction between cortical GLP-1 signaling and hippocampal neurotrophic pathways. In the AUD group, this relationship showed an inverse, although non-significant, trend, which could indicate a possible alteration in these cross-regional interactions in the context of chronic alcohol exposure. Although these findings should be interpreted cautiously, they suggest that the functional coupling between GLP-1 and BDNF pathways may differ between groups.

These differences may reflect alterations in the functional coupling between GLP-1 and BDNF pathways. Previous experimental evidence has suggested that GLP-1 signaling can influence BDNF expression and activity through intracellular pathways such as cAMP/CREB, which are involved in neuronal survival, synaptic plasticity, and neurogenesis[81-84]. For example, agonists of the GLP-1 receptor have been shown to upregulate BDNF and its downstream signaling cascades in neuronal cultures and animal models, contributing to neuroprotection and cognitive improvement in various pathological contexts, including models of neurodegeneration and psychiatric disorders[85,86]. Furthermore, GLP-1 and BDNF have been proposed to act synergistically to support cortical–hippocampal communication and regulate energy balance and stress responses in the brain[87-89].

Although the precise mechanisms underlying these interactions remain to be fully elucidated, the existing literature provides a biologically plausible framework linking GLP-1 signaling and BDNF pathways. The differences in gene–gene correlations observed between controls and individuals with AUD in the present study could reflect adaptive or maladaptive changes in these neurobiological systems associated with chronic alcohol exposure.

In summary, our results showed that chronic alcohol consumption, whether in humans or murine models, reduces GLP-1R gene expression in brain areas closely related to the reinforcing properties of ethanol. The simultaneous decrease in GLP-1R, OPRM-1, and BDNF across key mesocorticolimbic brain regions underscores a multifaceted neurobiological deficit in AUD. This finding opens new alternatives for further research into how this emerging receptor, GLP-1R, can be helpful as a potential biomarker for AUD, further supporting its therapeutic potential in this drug use disorder.

Limitations of the study

This study presents limitations. First, it was only conducted in males, suggesting that future studies should also assess potential alterations in GLP-1R gene expression in female subjects. Second, we measured gene expression but did not assess protein levels or receptor function. Finally, assessment of GLP-1R in other patterns of consumption (e.g., binge drinking) and ethanol relapse and abstinence will further contribute to characterizing the role of GLP-1R in AUD.

Acknowledgements

Tissues were received from the New South Wales Brain Tissue Resource Centre at the University of Sydney which is supported by the University of Sydney. Research reported in this publication was supported by the National Institute of Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Number R28AA012725. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

Data availability

The data generated during the current study are available from the corresponding author upon reasonable request.

Author Contributions

ABT performed gene expression analyses, acquired the data, assisted with data interpretation, and drafted the manuscript. MSGG assisted with data interpretation and formal analysis, and contributed to writing the original draft as well as reviewing and editing the final version. SOM conducted the linear regression and correlation analyses and provided statistical support. JM contributed to the study concept and design, and reviewed and edited the final version of the manuscript. All authors reviewed and approved the final version of the manuscript.

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations.

All animal procedures were approved by the Ethics and Research Integrity Committee of Miguel Hernández University (approval number: UMH.IN.JM.01.21) and were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals.

Human samples were obtained from the New South Wales Tissue Resource Centre (NSWBTRC) at the University of Sydney, Australia. The use of these samples was approved by the Ethics and Research Integrity Committee of Miguel Hernández University (approval number: IN-JM-001-11), in accordance with the biobank's governance procedures.

Written informed consent was obtained from all participants prior to sample collection.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by the INSTITUTO DE SALUD CARLOS III, SPANISH MINISTRY OF SCIENCE AND INNOVATION, grant number PI21/00488 to J.M. and RED DE INVESTIGACIÓN EN ATENCIÓN PRIMARIA DE ADICCIONES, INSTITUTO DE SALUD CARLOS III, SPANISH MINISTRY OF SCIENCE AND INNOVATION, grant number RD21/0009/0008 and RD24/0003/0002 to J.M., and Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL). The Instituto de Neurociencias is a “Centre of Excellence Severo Ochoa” (CEX2021-001165-S). ABT is a predoctoral fellow supported by Red de investigación en Atención Primaria de Adicciones (Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación, grant number RD21/0009/0008 and RD24/0003/0002).

References

1 Kranzler, H. R. Overview of Alcohol Use Disorder. *Am J Psychiatry* **180**, 565-572, doi:10.1176/appi.ajp.20230488 (2023).

2 Organization, W. H. (Geneva, 2024).

3 Fairbanks, J., Umbreit, A., Kolla, B. P., Karpyak, V. M., Schneekloth, T. D., Loukianova, L. L. *et al.* Evidence-Based Pharmacotherapies for Alcohol Use Disorder: Clinical Pearls. *Mayo Clin Proc* **95**, 1964-1977, doi:10.1016/j.mayocp.2020.01.030 (2020).

4 Liu, T., Shi, F., Guo, Z., Li, H. & Qin, D. Therapeutic Potential of the Novel GLP-1 Receptor Agonist Semaglutide in Alcohol Use Disorder. *Pharmacopsychiatry*, doi:10.1055/a-2550-6470 (2025).

5 Tanguturi Yella, S. S., Kota Sesha Brahma Sree, K. S. & Mahato, S. K. The Role of Glucagon-Like Peptide-1 Receptor Agonists in the Treatment of Alcohol Use Disorder: Current Evidence and Future Directions. *J Clin Psychopharmacol* **45**, 372-375, doi:10.1097/JCP.0000000000002010 (2025).

6 Graaf, C., Donnelly, D., Wootten, D., Lau, J., Sexton, P. M., Miller, L. J. *et al.* Glucagon-Like Peptide-1 and Its Class B G Protein-Coupled Receptors: A Long March to Therapeutic Successes. *Pharmacol Rev* **68**, 954-1013, doi:10.1124/pr.115.011395 (2016).

7 Smith, N. K., Hackett, T. A., Galli, A. & Flynn, C. R. GLP-1: Molecular mechanisms and outcomes of a complex signaling system. *Neurochem Int* **128**, 94-105, doi:10.1016/j.neuint.2019.04.010 (2019).

8 Zaimia, N., Obeid, J., Varrault, A., Sabatier, J., Broca, C., Gilon, P. *et al.* GLP-1 and GIP receptors signal through distinct beta-arrestin 2-dependent pathways to regulate pancreatic beta cell function. *Cell Rep* **42**, 113326, doi:10.1016/j.celrep.2023.113326 (2023).

9 Al-Noshokaty, T. M., Abdelhamid, R., Abdelmaksoud, N. M., Khaled, A., Hossam, M., Ahmed, R. *et al.* Unlocking the multifaceted roles of GLP-1: Physiological functions and therapeutic potential. *Toxicol Rep* **14**, 101895, doi:10.1016/j.toxrep.2025.101895 (2025).

10 Cork, S. C., Richards, J. E., Holt, M. K., Gribble, F. M., Reimann, F. & Trapp, S. Distribution and characterisation of Glucagon-like peptide-1 receptor expressing cells in the mouse brain. *Mol Metab* **4**, 718-731, doi:10.1016/j.molmet.2015.07.008 (2015).

11 Richards, P., Parker, H. E., Adriaenssens, A. E., Hodgson, J. M., Cork, S. C., Trapp, S. *et al.* Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes* **63**, 1224-1233, doi:10.2337/db13-1440 (2014).

12 Diz-Chaves, Y., Herrera-Perez, S., Gonzalez-Matias, L. C., Lamas, J. A. & Mallo, F. Glucagon-Like Peptide-1 (GLP-1) in the Integration of Neural and Endocrine Responses to Stress. *Nutrients* **12**, doi:10.3390/nu12113304 (2020).

13 Merkel, R., Hernandez, N. S., Weir, V., Zhang, Y., Caffrey, A., Rich, M. T. *et al.* An endogenous GLP-1 circuit engages VTA GABA neurons to regulate mesolimbic dopamine neurons and attenuate cocaine seeking. *Sci Adv* **11**, eadr5051, doi:10.1126/sciadv.adr5051 (2025).

14 Egecioglu, E., Steensland, P., Fredriksson, I., Feltmann, K., Engel, J. A. & Jerlhag, E. The glucagon-like peptide 1 analogue Exendin-4 attenuates alcohol mediated behaviors in rodents. *Psychoneuroendocrinology* **38**, 1259-1270, doi:10.1016/j.psyneuen.2012.11.009 (2013).

15 Shirazi, R. H., Dickson, S. L. & Skibicka, K. P. Gut peptide GLP-1 and its analogue, Exendin-4, decrease alcohol intake and reward. *PLoS One* **8**, e61965, doi:10.1371/journal.pone.0061965 (2013).

16 Marty, V. N., Farokhnia, M., Munier, J. J., Mulpuri, Y., Leggio, L. & Spigelman, I. Long-Acting Glucagon-Like Peptide-1 Receptor Agonists Suppress Voluntary Alcohol Intake in Male Wistar Rats. *Front Neurosci* **14**, 599646, doi:10.3389/fnins.2020.599646 (2020).

17 Aranas, C., Edvardsson, C. E., Shevchouk, O. T., Zhang, Q., Witley, S., Blid Skoldheden, S. *et al.* Semaglutide reduces alcohol intake and relapse-like drinking in male and female rats. *EBioMedicine* **93**, 104642, doi:10.1016/j.ebiom.2023.104642 (2023).

18 Tang-Christensen, M., Larsen, P. J., Goke, R., Fink-Jensen, A., Jessop, D. S., Moller, M. *et al.* Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. *Am J Physiol* **271**, R848-856, doi:10.1152/ajpregu.1996.271.4.R848 (1996).

19 Navarro, M., Rodriquez de Fonseca, F., Alvarez, E., Chowen, J. A., Zueco, J. A., Gomez, R. *et al.* Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J Neurochem* **67**, 1982-1991, doi:10.1046/j.1471-4159.1996.67051982.x (1996).

20 Thomsen, M., Dencker, D., Wortwein, G., Weikop, P., Egecioglu, E., Jerlhag, E. *et al.* The glucagon-like peptide 1 receptor agonist Exendin-4 decreases relapse-like drinking in socially housed mice. *Pharmacol Biochem Behav* **160**, 14-20, doi:10.1016/j.pbb.2017.07.014 (2017).

21 Edvardsson, C. E., Cadeddu, D., Ericson, M., Adermark, L. & Jerlhag, E. An inhibitory GLP-1 circuit in the lateral septum modulates reward processing and alcohol intake in rodents. *EBioMedicine* **115**, 105684, doi:10.1016/j.ebiom.2025.105684 (2025).

22 Sorensen, G., Caine, S. B. & Thomsen, M. Effects of the GLP-1 Agonist Exendin-4 on Intravenous Ethanol Self-Administration in Mice. *Alcohol Clin Exp Res* **40**, 2247-2252, doi:10.1111/acer.13199 (2016).

23 Klausen, M. K., Jensen, M. E., Moller, M., Le Dous, N., Jensen, A. O., Zeeman, V. A. *et al.* Exenatide once weekly for alcohol use disorder investigated in a randomized, placebo-controlled clinical trial. *JCI Insight* **7**, doi:10.1172/jci.insight.159863 (2022).

24 Richards, J. R., Dorand, M. F., Royal, K., Mnajjed, L., Paszkowiak, M. & Simmons, W. K. Significant Decrease in Alcohol Use Disorder Symptoms Secondary to Semaglutide Therapy for Weight Loss: A Case Series. *J Clin Psychiatry* **85**, doi:10.4088/JCP.23m15068 (2023).

25 Quddos, F., Hubshman, Z., Tegge, A., Sane, D., Marti, E., Kablinger, A. S. *et al.* Semaglutide and Tirzepatide reduce alcohol consumption in individuals with obesity. *Sci Rep* **13**, 20998, doi:10.1038/s41598-023-48267-2 (2023).

26 Farokhnia, M., Fede, S. J., Grodin, E. N., Browning, B. D., Crozier, M. E., Schwandt, M. L. *et al.* Differential association between the GLP1R gene variants and brain functional connectivity according to the severity of alcohol use. *Sci Rep* **12**, 13027, doi:10.1038/s41598-022-17190-3 (2022).

27 Suchankova, P., Yan, J., Schwandt, M. L., Stangl, B. L., Caparelli, E. C., Momenan, R. *et al.* The glucagon-like peptide-1 receptor as a potential treatment target in alcohol use disorder: evidence from human genetic association studies and a mouse model of alcohol dependence. *Transl Psychiatry* **5**, e583, doi:10.1038/tp.2015.68 (2015).

28 Vallof, D., Kalafateli, A. L. & Jerlhag, E. Brain region specific glucagon-like peptide-1 receptors regulate alcohol-induced behaviors in rodents. *Psychoneuroendocrinology* **103**, 284-295, doi:10.1016/j.psyneuen.2019.02.006 (2019).

29 Yang, Y., Tong, M. & de la Monte, S. M. Early-Stage Moderate Alcohol Feeding Dysregulates Insulin-Related Metabolic Hormone Expression in the Brain: Potential Links to Neurodegeneration Including Alzheimer's Disease. *J Alzheimers Dis Rep* **8**, 1211-1228, doi:10.3233/ADR-240026 (2024).

30 Marquez-Meneses, J. D., Olaya-Bonilla, S. A., Barrera-Carreno, S., Tibaduiza-Arevalo, L. C., Forero-Cardenas, S., Carrillo-Vaca, L. *et al.* GLP-1 Analogues in the Neurobiology of Addiction: Translational Insights and Therapeutic Perspectives. *Int J Mol Sci* **26**, doi:10.3390/ijms26115338 (2025).

31 Farokhnia, M., Browning, B. D., Crozier, M. E., Sun, H., Akhlaghi, F. & Leggio, L. The glucagon-like peptide-1 system is modulated by acute and chronic alcohol exposure: Findings from human laboratory experiments and a post-mortem brain study. *Addict Biol* **27**, e13211, doi:10.1111/adb.13211 (2022).

32 Thorsell, A. The mu-opioid receptor and treatment response to naltrexone. *Alcohol Alcohol* **48**, 402-408, doi:10.1093/alcalc/agt030 (2013).

33 Hansson, A. C., Grunder, G., Hirth, N., Noori, H. R., Spanagel, R. & Sommer, W. H. Dopamine and opioid systems adaptation in alcoholism revisited: Convergent evidence from positron emission tomography and postmortem studies. *Neurosci Biobehav Rev* **106**, 141-164, doi:10.1016/j.neubiorev.2018.09.010 (2019).

34 Berrettini, W. Opioid neuroscience for addiction medicine: From animal models to FDA approval for alcohol addiction. *Prog Brain Res* **223**, 253-267, doi:10.1016/bs.pbr.2015.07.030 (2016).

35 Cutuli, D. & Sampedro-Piquero, P. BDNF and its Role in the Alcohol Abuse Initiated During Early Adolescence: Evidence from Preclinical and Clinical Studies. *Curr Neuropharmacol* **20**, 2202-2220, doi:10.2174/1570159X20666220624111855 (2022).

36 Ornell, F., Hansen, F., Schuch, F. B., Pezzini Rebelatto, F., Tavares, A. L., Scherer, J. N. *et al.* Brain-derived neurotrophic factor in substance use disorders: A systematic review and meta-analysis. *Drug Alcohol Depend* **193**, 91-103, doi:10.1016/j.drugalcdep.2018.08.036 (2018).

37 Haerian, B. S. BDNF rs6265 polymorphism and drug addiction: a systematic review and meta-analysis. *Pharmacogenomics* **14**, 2055-2065, doi:10.2217/pgs.13.217 (2013).

38 Oomen, C. A., Bekinschtein, P., Kent, B. A., Saksida, L. M. & Bussey, T. J. Adult hippocampal neurogenesis and its role in cognition. *Wiley Interdiscip Rev Cogn Sci* **5**, 573-587, doi:10.1002/wcs.1304 (2014).

39 Goto, A. Synaptic plasticity during systems memory consolidation. *Neurosci Res* **183**, 1-6, doi:10.1016/j.neures.2022.05.008 (2022).

40 Chen, G., Lai, S., Bao, G., Ke, J., Meng, X., Lu, S. *et al.* Distinct reward processing by subregions of the nucleus accumbens. *Cell Rep* **42**, 112069, doi:10.1016/j.celrep.2023.112069 (2023).

41 Klawonn, A. M. & Malenka, R. C. Nucleus Accumbens Modulation in Reward and Aversion. *Cold Spring Harb Symp Quant Biol* **83**, 119-129, doi:10.1101/sqb.2018.83.037457 (2018).

42 Ortega-Alvaro, A., Ternianov, A., Aracil-Fernandez, A., Navarrete, F., Garcia-Gutierrez, M. S. & Manzanares, J. Role of cannabinoid CB2 receptor in the reinforcing actions of ethanol. *Addict Biol* **20**, 43-55, doi:10.1111/adb.12076 (2015).

43 Viudez-Martinez, A., Garcia-Gutierrez, M. S., Navarron, C. M., Morales-Calero, M. I., Navarrete, F., Torres-Suarez, A. I. *et al.* Cannabidiol reduces ethanol consumption, motivation and relapse in mice. *Addict Biol* **23**, 154-164, doi:10.1111/adb.12495 (2018).

44 KBJ, P. G. a. F. *Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates*. 5th edn, (Academic Press, 2019).

45 Palkovits, M. Punch sampling biopsy technique. *Methods Enzymol* **103**, 368-376, doi:10.1016/s0076-6879(83)03025-6 (1983).

46 Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408, doi:10.1006/meth.2001.1262 (2001).

47 Sutherland, G. T., Sheedy, D., Stevens, J., McCrossin, T., Smith, C. C., van Roijen, M. *et al.* The NSW brain tissue resource centre: Banking for alcohol and major neuropsychiatric disorders research. *Alcohol* **52**, 33-39, doi:10.1016/j.alcohol.2016.02.005 (2016).

48 Merchenthaler, I., Lane, M. & Shughrue, P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol* **403**, 261-280, doi:10.1002/(sici)1096-9861(19990111)403:2<261::aid-cne8>3.0.co;2-5 (1999).

49 Jensen, C. B., Pyke, C., Rasch, M. G., Dahl, A. B., Knudsen, L. B. & Secher, A. Characterization of the Glucagonlike Peptide-1 Receptor in Male Mouse Brain Using a

Novel Antibody and In Situ Hybridization. *Endocrinology* **159**, 665-675, doi:10.1210/en.2017-00812 (2018).

50 Graham, D. L., Durai, H. H., Trammell, T. S., Noble, B. L., Mortlock, D. P., Galli, A. *et al.* A novel mouse model of glucagon-like peptide-1 receptor expression: A look at the brain. *J Comp Neurol* **528**, 2445-2470, doi:10.1002/cne.24905 (2020).

51 Dixon, T. N., McNally, G. P. & Ong, Z. Y. Glucagon-Like Peptide-1 Receptor Signaling in the Ventral Tegmental Area Reduces Alcohol Self-Administration in Male Rats. *Alcohol Clin Exp Res* **44**, 2118-2129, doi:10.1111/acer.14437 (2020).

52 Allingbjerg, M. L., Hansen, S. N., Secher, A. & Thomsen, M. Glucagon-like peptide-1 receptors in nucleus accumbens, ventral hippocampus, and lateral septum reduce alcohol reinforcement in mice. *Exp Clin Psychopharmacol* **31**, 612-620, doi:10.1037/pha0000620 (2023).

53 Vallof, D., Maccioni, P., Colombo, G., Mandrapa, M., Jornulf, J. W., Egecioglu, E. *et al.* The glucagon-like peptide 1 receptor agonist liraglutide attenuates the reinforcing properties of alcohol in rodents. *Addict Biol* **21**, 422-437, doi:10.1111/adb.12295 (2016).

54 Vallof, D., Kalafateli, A. L. & Jerlhag, E. Long-term treatment with a glucagon-like peptide-1 receptor agonist reduces ethanol intake in male and female rats. *Transl Psychiatry* **10**, 238, doi:10.1038/s41398-020-00923-1 (2020).

55 Chuong, V., Farokhnia, M., Khom, S., Pince, C. L., Elvig, S. K., Vlkolinsky, R. *et al.* The glucagon-like peptide-1 (GLP-1) analogue semaglutide reduces alcohol drinking and modulates central GABA neurotransmission. *JCI Insight* **8**, doi:10.1172/jci.insight.170671 (2023).

56 Fink-Jensen, A., Wortwein, G., Klausen, M. K., Holst, J. J., Hartmann, B., Thomsen, M. *et al.* Effect of the glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide on alcohol consumption in alcohol-preferring male vervet monkeys. *Psychopharmacology (Berl)* **242**, 63-70, doi:10.1007/s00213-024-06637-2 (2025).

57 Urkon, M., Ferencz, E., Szasz, J. A., Szabo, M. I. M., Orban-Kis, K., Szatmari, S. *et al.* Antidiabetic GLP-1 Receptor Agonists Have Neuroprotective Properties in Experimental Animal Models of Alzheimer's Disease. *Pharmaceuticals (Basel)* **18**, doi:10.3390/ph18050614 (2025).

58 Si, J., Yu, K., Hao, J., Wang, J. & Zhang, L. The therapeutic effects and mechanisms of glucagon-like peptide-1 receptor agonists in neurocognitive disorders. *Ther Adv Neurol Disord* **18**, 17562864251332035, doi:10.1177/17562864251332035 (2025).

59 Kenzheshova, A., Moldasheva, A. & Aljofan, M. Neuroprotective Role of the Novel GLP-1R Agonist Semaglutide. *Curr Med Chem*, doi:10.2174/0109298673359635250401051339 (2025).

60 Zhang, L. Q., Zhang, W., Li, T., Yang, T., Yuan, X., Zhou, Y. *et al.* GLP-1R activation ameliorated novel-object recognition memory dysfunction via regulating hippocampal AMPK/NF-kappaB pathway in neuropathic pain mice. *Neurobiol Learn Mem* **182**, 107463, doi:10.1016/j.nlm.2021.107463 (2021).

61 Chaves Filho, A. J. M., Cunha, N. L., de Souza, A. G., Soares, M. V., Juca, P. M., de Queiroz, T. *et al.* The GLP-1 receptor agonist liraglutide reverses mania-like alterations and memory deficits induced by D-amphetamine and augments lithium effects in mice:

Relevance for bipolar disorder. *Prog Neuropsychopharmacol Biol Psychiatry* **99**, 109872, doi:10.1016/j.pnpbp.2020.109872 (2020).

62 Tabakoff, B. & Hoffman, P. L. Alcohol interactions with brain opiate receptors. *Life Sci* **32**, 197-204, doi:10.1016/0024-3205(83)90031-0 (1983).

63 Levine, A. S., Hess, S. & Morley, J. E. Alcohol and the opiate receptor. *Alcohol Clin Exp Res* **7**, 83-84, doi:10.1111/j.1530-0277.1983.tb05416.x (1983).

64 Hoffman, P. L., Chung, C. T. & Tabakoff, B. Effects of ethanol, temperature, and endogenous regulatory factors on the characteristics of striatal opiate receptors. *J Neurochem* **43**, 1003-1010, doi:10.1111/j.1471-4159.1984.tb12836.x (1984).

65 Tabakoff, B., Urwyler, S. & Hoffman, P. L. Ethanol alters kinetic characteristics and function of striatal morphine receptors. *J Neurochem* **37**, 518-521, doi:10.1111/j.1471-4159.1981.tb00487.x (1981).

66 Hoffman, P. L., Urwyler, S. & Tabakoff, B. Alterations in opiate receptor function after chronic ethanol exposure. *J Pharmacol Exp Ther* **222**, 182-189 (1982).

67 Flores-Gomez, M., Cantero-Garcia, N., Pineda-Gomez, J. P., Moh-Ahmed, A., Flores-Burgess, A., Diaz-Cabiale, Z. *et al.* Galanin(1-15) and Naltrexone: A novel approach for alcohol use disorder in rats, involving the mesolimbic system. *Biomed Pharmacother* **188**, 118170, doi:10.1016/j.biopha.2025.118170 (2025).

68 Carvour, H. M., Roemer, C., Underwood, D. P., Padilla, E. S., Sandoval, O., Robertson, M. *et al.* Mu-opioid receptor knockout on Foxp2-expressing neurons reduces aversion-resistant alcohol drinking. *Pharmacol Biochem Behav* **247**, 173932, doi:10.1016/j.pbb.2024.173932 (2025).

69 Turchan, J., Przewlocka, B., Toth, G., Lason, W., Borsodi, A. & Przewlocki, R. The effect of repeated administration of morphine, cocaine and ethanol on mu and delta opioid receptor density in the nucleus accumbens and striatum of the rat. *Neuroscience* **91**, 971-977, doi:10.1016/s0306-4522(98)00637-x (1999).

70 Gianoulakis, C. & de Waele, J. P. Genetics of alcoholism: role of the endogenous opioid system. *Metab Brain Dis* **9**, 105-131, doi:10.1007/BF01999765 (1994).

71 Weerts, E. M., McCaul, M. E., Kuwabara, H., Yang, X., Xu, X., Dannals, R. F. *et al.* Influence of OPRM1 Asn40Asp variant (A118G) on [11C]carfentanil binding potential: preliminary findings in human subjects. *Int J Neuropsychopharmacol* **16**, 47-53, doi:10.1017/S146114571200017X (2013).

72 Hou, J., Deng, Q., Wu, J. & Chen, W. Adolescent intermittent alcohol exposure induces adult cognitive deficits via disrupting the septo-hippocampal cholinergic projections. *Life Sci* **377**, 123795, doi:10.1016/j.lfs.2025.123795 (2025).

73 Arioli, M., Bossert, I., D'Ambrosio, D., Manera, M., Andreolli, E. M., Canessa, N. *et al.* Neural correlates of executive dysfunction in alcohol use disorder: preliminary evidence from (18)F-FDG-PET. *Front Psychol* **16**, 1568085, doi:10.3389/fpsyg.2025.1568085 (2025).

74 Cheng, Y., Magnard, R., Langdon, A. J., Lee, D. & Janak, P. H. Chronic ethanol exposure produces sex-dependent impairments in value computations in the striatum. *Sci Adv* **11**, eadt0200, doi:10.1126/sciadv.adt0200 (2025).

75 Garcia-Dolores, F., Hernandez-Torres, M. A., Fuentes-Medel, E., Diaz, A., Guevara, J., Baltazar-Gaytan, E. *et al.* Atrophy and Higher Levels of Inflammatory-Related Markers in

the Posterior Cerebellar Lobe Cortex in Chronic Alcohol Use Disorder: A Cross-Sectional Study. *Neuropathol Appl Neurobiol* **51**, e70011, doi:10.1111/nan.70011 (2025).

76 Matthews, D. B., Kerr, E., Zank, A., Hartwig, J., Garscia, A., Stumo, S. *et al.* Recent Investigations Designed to Unravel the Interaction of Age and Alcohol on Behavior and Cognition: Potential Neurobiological Mechanisms. *Adv Exp Med Biol* **1473**, 243-256, doi:10.1007/978-3-031-81908-7_11 (2025).

77 Liu, S., Xie, X., Zhao, D., Jin, N., Hu, Y., Wang, W. *et al.* Alcohol use disorder disrupts BDNF maturation via the PAI-1 pathway which could be reversible with abstinence. *Sci Rep* **14**, 22150, doi:10.1038/s41598-024-73347-2 (2024).

78 Malewska-Kasprzak, M., Skibinska, M. & Dmitrzak-Weglarz, M. Alterations in Neurotrophins in Alcohol-Addicted Patients during Alcohol Withdrawal. *Brain Sci* **14**, doi:10.3390/brainsci14060583 (2024).

79 Nieto, S. J., Haile, C. N., Quave, C. B., Harding, M. J., Nielsen, D. A., Meisch, R. A. *et al.* Paternal alcohol exposure reduces acquisition of operant alcohol self-administration and affects Bdnf DNA methylation in male and female offspring. *Addict Biol* **27**, e13078, doi:10.1111/adb.13078 (2022).

80 Hogan, N. L., Jaehne, E. J., Bak, S., Djouma, E. & van den Buuse, M. Brain-Derived neurotrophic factor Val66Met induces female-specific changes in impulsive behaviour and alcohol self-administration in mice. *Behav Brain Res* **401**, 113090, doi:10.1016/j.bbr.2020.113090 (2021).

81 Perry, T., Lahiri, D. K., Chen, D., Zhou, J., Shaw, K. T., Egan, J. M. *et al.* A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. *J Pharmacol Exp Ther* **300**, 958-966, doi:10.1124/jpet.300.3.958 (2002).

82 Velmurugan, K., Bouchard, R., Mahaffey, G. & Pugazhenthi, S. Neuroprotective actions of glucagon-like peptide-1 in differentiated human neuroprogenitor cells. *J Neurochem* **123**, 919-931, doi:10.1111/jnc.12036 (2012).

83 Ohtake, N., Saito, M., Eto, M. & Seki, K. Exendin-4 promotes the membrane trafficking of the AMPA receptor GluR1 subunit and ADAM10 in the mouse neocortex. *Regul Pept* **190-191**, 1-11, doi:10.1016/j.regpep.2014.04.003 (2014).

84 Gumuslu, E., Mutlu, O., Celikyurt, I. K., Ulak, G., Akar, F., Erden, F. *et al.* Exenatide enhances cognitive performance and upregulates neurotrophic factor gene expression levels in diabetic mice. *Fundam Clin Pharmacol* **30**, 376-384, doi:10.1111/fcp.12192 (2016).

85 Detka, J. & Glombik, K. Insights into a possible role of glucagon-like peptide-1 receptor agonists in the treatment of depression. *Pharmacol Rep* **73**, 1020-1032, doi:10.1007/s43440-021-00274-8 (2021).

86 Siddeeque, N., Hussein, M. H., Abdelmaksoud, A., Bishop, J., Attia, A. S., Elshazli, R. M. *et al.* Neuroprotective effects of GLP-1 receptor agonists in neurodegenerative Disorders: A Large-Scale Propensity-Matched cohort study. *Int Immunopharmacol* **143**, 113537, doi:10.1016/j.intimp.2024.113537 (2024).

87 Ma, Q., Wang, L., Liu, X. X., An, Z. G., Luo, X., Zhang, L. L. *et al.* GLP-1 plays a protective role in hippocampal neuronal cells by activating cAMP-CREB-BDNF signaling pathway

against CORT+HG-induced toxicity. *Heliyon* **9**, e18491, doi:10.1016/j.heliyon.2023.e18491 (2023).

88 Reich, N. & Holscher, C. The neuroprotective effects of glucagon-like peptide 1 in Alzheimer's and Parkinson's disease: An in-depth review. *Front Neurosci* **16**, 970925, doi:10.3389/fnins.2022.970925 (2022).

89 Liu, Y., Hu, Z., Wang, J., Liao, Y. & Shu, L. Puerarin alleviates depressive-like behaviors in high-fat diet-induced diabetic mice via modulating hippocampal GLP-1R/BDNF/TrkB signaling. *Nutr Neurosci* **26**, 997-1010, doi:10.1080/1028415X.2022.2112439 (2023).

Figure and legends

Figure 1. Total RNA integrity number (RIN) evaluation in the prefrontal cortex (PFC), nucleus accumbens (NAc) and hippocampus (HIP) of AUD subjects (n=18) and controls (n=18). Representative electropherograms and gel images of the mean RIN value from LPFC (A), NAc (B) and HIP (C) brain regions. (D) The grey box contains the global mean value from all the studied samples in the RIN values summary table.

Figure 2. Voluntary ethanol consumption in male C57Bl/6J mice. A) Ethanol intake is measured in g/kg/day, with data collected on the volume consumed every 24 hours. B) The preference for ethanol intake is calculated as the ratio of ethanol consumption to total intake [ethanol preference = ethanol consumption/(ethanol consumption + water consumption)]. The dots indicate the means, with vertical lines representing the ±SEM for each parameter assessed.

Figure 3. Relative gene expression of GLP-1R, OPRM1 and BDNF in mesocorticolimbic brain areas of mice exposed to chronic voluntary ethanol consumption. A) Changes in the relative gene expression of GLP-1R in the prefrontal cortex (PFC), B) nucleus accumbens (NAc), and C) hippocampus (HIP) of mice exposed to the two-bottle choice test. D) Changes in the relative gene expression of OPRM1 in the NAc of mice exposed to the two-bottle choice test. E)

Changes in the relative gene expression of BDNF in the HIP of mice exposed to the two-bottle choice test. The dots indicate the means, with vertical lines representing the ±SEM for each parameter assessed. *Indicates values from mice exposed to VEC that significantly differ from the vehicle group.

Figure 4. Relative gene expression of GLP-1R, OPRM1 and BDNF in mesocorticolimbic brain areas of patients with AUD. A) Changes in the relative gene expression of GLP-1R in the prefrontal cortex (PFC), B) nucleus accumbens (NAc), and C) hippocampus (HIP) of individuals with AUD. D) Changes in the relative gene expression of OPRM1 in the NAc of individuals with AUD. E) Changes in the relative gene expression of BDNF in the HIP of individuals with AUD. The dots indicate the means, with vertical lines representing the ±SEM for each parameter assessed. *Indicates values from AUD patients that significantly differ from controls.

Table 1. Demographic and clinical data of patients with AUD and their corresponding control group

Subject	Age	PMI (hours)	Brain pH	Began drinking	Drinking years	COD category	Clinical History	Smoking	BMI
AUD1	67	48	6.40	25	42	Respiratory/Toxicity	None	Current	21.5
AUD2	41	54	6.70	25	16	Neurological	None	Current	21.7
AUD3	64	39	6.76	25	39	Toxicity	Depression	Current	20.3
AUD4	45	18.5	6.57	14	31	Respiratory	Depression	Current	29.1
AUD5	65	7	6.47	25	40	Stroke	Depression	Current	20
AUD6	61	59	6.57	16	45	Cardiac	Depression	Current	25.2
AUD7	49	44	6.41	16	33	Cardiac	None	Current	25.8
AUD8	49	16	6.19	14	35	Cardiac	None	Current	32.7
AUD9	62	40	6.59	25	37	Cardiac	Depression	Ex-smoker	34.6
AUD10	44	59	6.87	18	26	Cardiac	Depression	Current	24.5
AUD11	60	28	6.48	17	43	Infection	None	Current	42.3
AUD12	50	34.5	6.93	16	34	Respiratory/Toxicity	Depression	Ex-smoker	28.2
AUD13	70	62	6.82	25	45	Cardiac	Depression	Current	26.8
AUD14	43	29	6.29	25	18	Hepatic/Blood Loss	None	Current	31.8
AUD15	58	21.5	6.65	20	38	Infection	None	Current	23.9
AUD16	55	17	6.85	25	30	Respiratory	Depression	Never	29.7
AUD17	58	44.5	6.47	15	43	Cardiac	None	Current	19.9

AUD18	63	28	6.89	19	44	Respiratory	None	Current	19.2
C1	50	30	6.37	-	-	Cardiac	None	Current	28.2
C2	59	40	6.53	-	-	Cardiac	None	Ex-smoker	25.8
C3	55	12	6.39	-	-	Cardiac	None	Never	28.7
C4	50	40	6.87	-	-	Cardiac	None	Current	28.6
C5	69	52	6.95	-	-	Cardiac	None	Never	21.6
C6	67	25	6.70	-	-	Cardiac	None	Ex-smoker	32.4
C7	48	17	6.62	-	-	Cardiac	None	Never	33.2
C8	59	28	6.77	-	-	Cardiac	None	Current	35
C9	64	41	6.73	-	-	Cardiac	None	Never	25.3
C10	47	27	6.66	-	-	Cardiac	None	Current	29.2
C11	61	22	6.41	-	-	Cardiac	None	Current	29.9
C12	61	30	6.69	-	-	Cardiac	None	Never	34.5
C13	66	32	6.66	-	-	Cardiac	None	Never	33.7
C14	53	26	6.36	-	-	Cardiac	None	Ex-smoker	29.6
C15	66	63	6.91	-	-	Cardiac	None	Never	27.5
C16	62	46	6.95	-	-	Cardiac	None	Ex-smoker	32.8
C17	37	14.5	6.46	-	-	Cardiac	None	Never	24.7
C18	40	27	6.79	-	-	Cardiac	None	Never	34.9

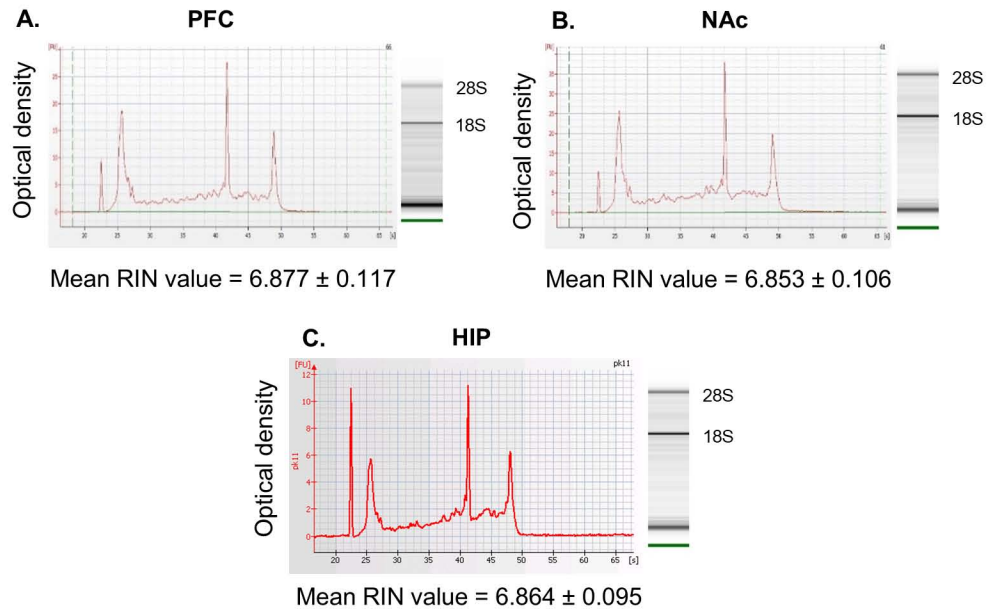
AUD: alcohol use disorder; C: controls; PMI: postmortem interval; COD: cause of death.

Table 2. Pearson's and Spearman's rank correlation coefficients for GLP-1R, OPRM1, BDNF gene expressions and age, PMI, pH, RIN, Began Drinking, Drinking years for controls and AUD cases

			Age	PMI	pH	RIN	BMI	Began Drinking	Drinking Years
PFC	**C**	**GLP-1R**	r= -0.07** p= 0.82	r= -0.28** p= 0.34	r= -0.18** p= 0.55	r= -0.2** p= 0.5	r=0.02** p=0.95		
	AUD	**GLP-1R**	r= 0.12** p= 0.62	**r= 0.71** p= <0.01**	r= -0.32** p= 0.19	r= -0.25** p= 0.31	r=0.04** p=0.87	r= -0.1** p= 0.7	r= 0.33** p=0.19
NAc	**C**	**GLP-1R**	r= 0.41 p= 0.14	r= 0.14 p= 0.64	r= 0.06 p= 0.85	r= -0.26 p= 0.36	r=-0.10 p=0.74		
	AUD	**GLP-1R**	r= -0.14 p= 0.59	r= 0.26 p= 0.33	r= 0.29 p= 0.28	r= -0.39 p= 0.14	r=-0.20 p=0.45	r= -0.03** p= 0.91	r= -0.07** p= 0.79
	C	**OPRM1**	r= -0.41 p= 0.11	r= -0.09 p= 0.73	r= 0.01 p= 0.96	r= 0.19 p= 0.47	r=-0.15 p=0.59		
	AUD	**OPMR1**	r= -0.21 p= 0.46	r= 0.23 p= 0.41	r= -0.13 p= 0.65	r= 0.26 p= 0.34	r= 0.01 p=0.96	r= 0.27** p= 0.33	r= -0.34** p=0.21
HIP	**C**	**GLP-1R**	r= 0.48 p= 0.08	r= 0.26 p= 0.37	r= 0.42 p= 0.13	r= 0.42 p= 0.14	r=0.31 p=0.27		
	AUD	**GLP-1R**	r= 0.15 p= 0.54	r= -0.11 p= 0.67	r= 0.45 p= 0.06	r= -0.24 p= 0.34	r=-0.27 p=0.28	r= 0.28** p= 0.25	r=0.01** p= 0.97
	C	**BDNF**	r= -0.47 p= 0.09	r= -0.08 p= 0.79	r= 0.03 p= 0.92	r= 0.12 p= 0.68	r=0.09 p=0.77		
	AUD	**BDNF**	r= -0.04 p=0.87	r= -0.14 p= 0.57	r= -0.17 p= 0.51	r= -0.28 p= 0.26	r=0.02 p=0.92	r= 0.27** p= 0.29	r= -0.24** p= 0.33

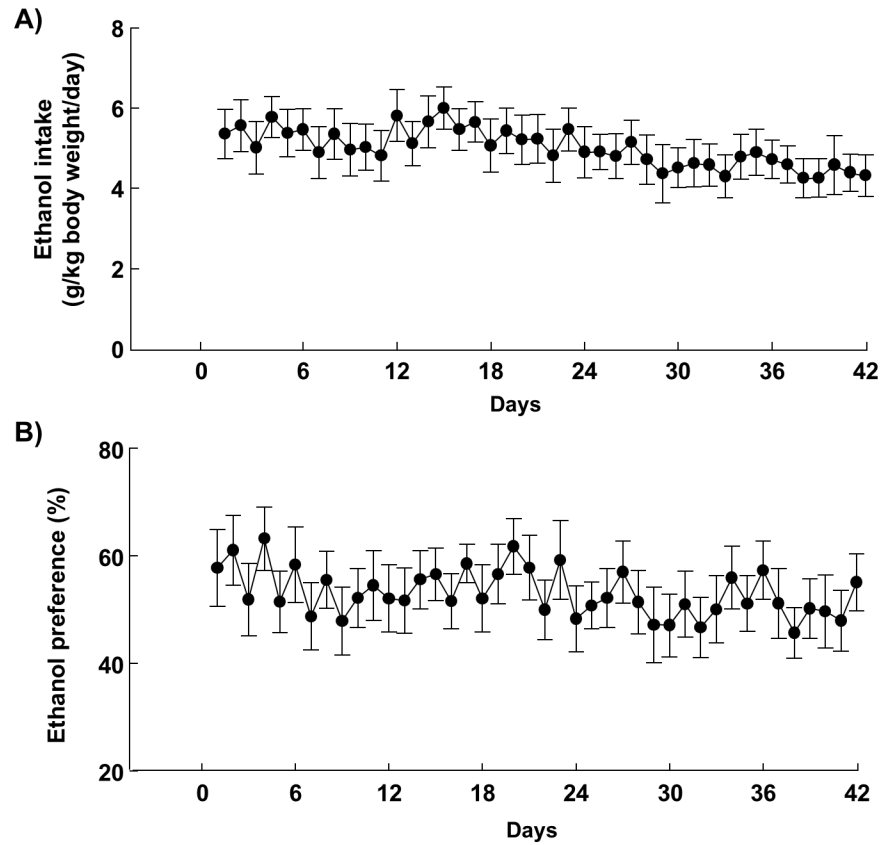
C: controls; AUD: alcohol use disorder; GLP-1R: glucagon-like peptide-1 receptor; PMI: postmortem interval; RIN: RNA integrity number.

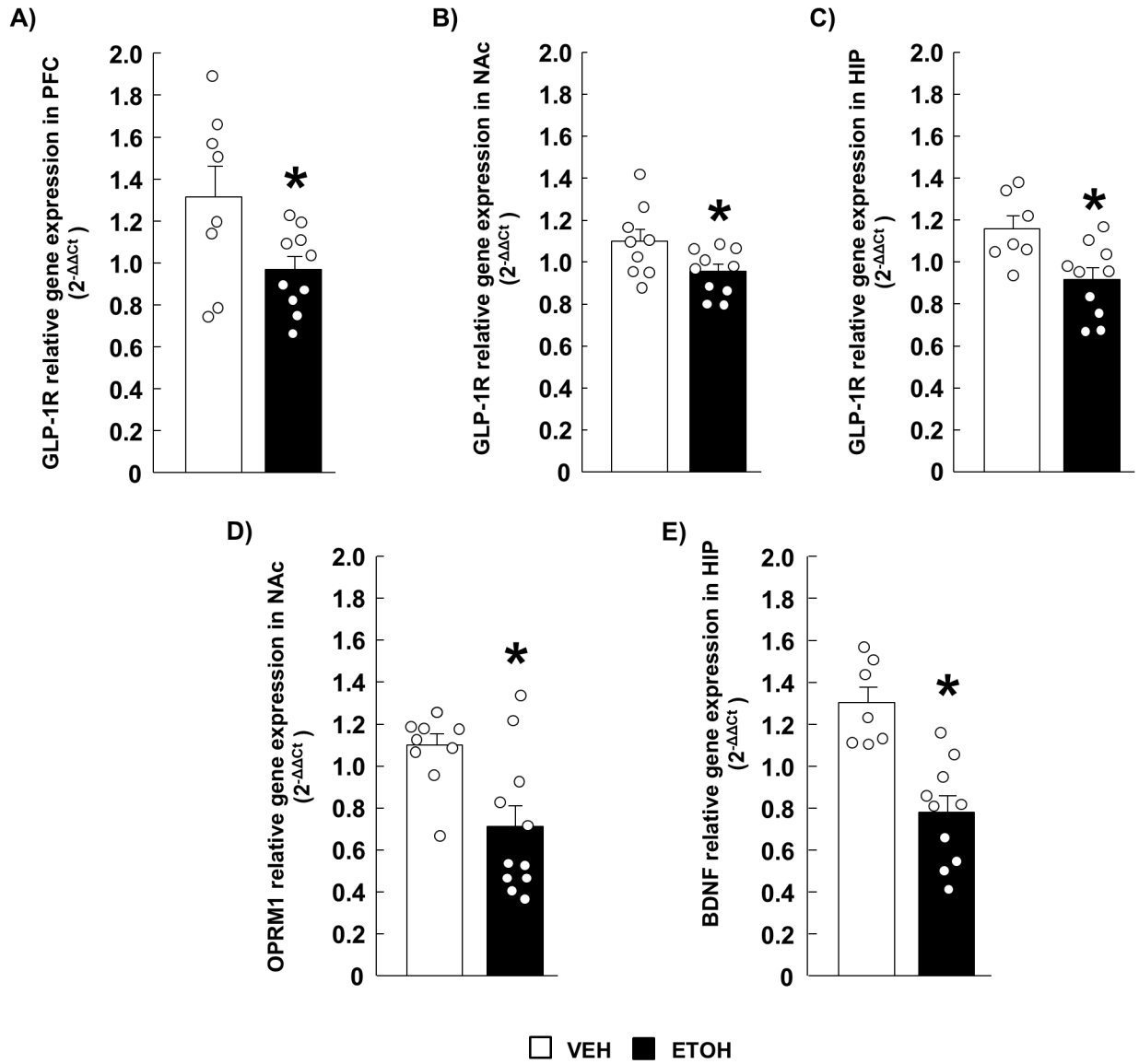
For normally distributed numeric variables, correlations were assessed using Pearson's correlation coefficient, while Spearman's rank correlation coefficient was applied for non-normally distributed variables (**).

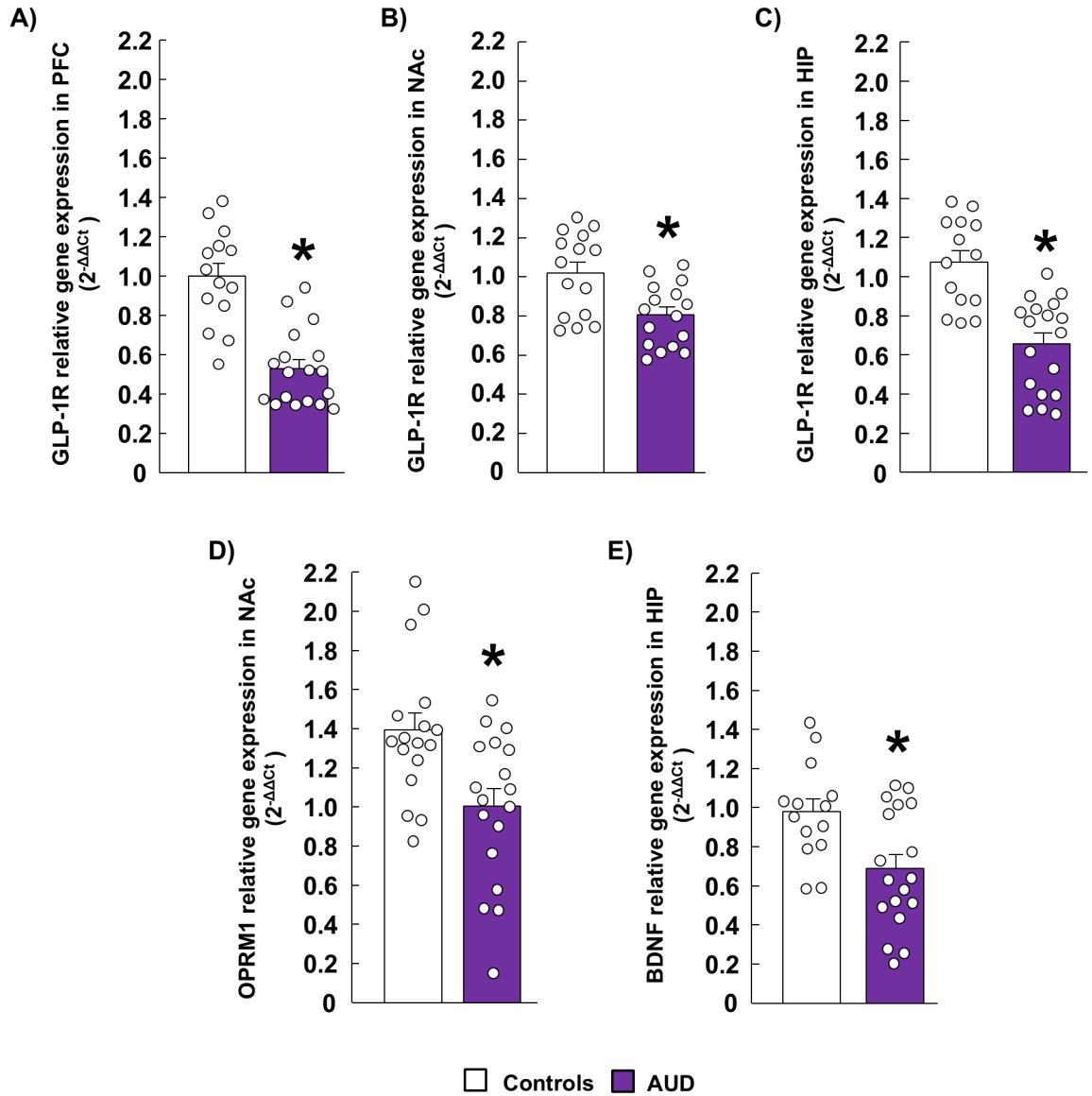


D. RIN values summary (mean ± SEM)

	DLPFC	NAc	HIP	*Mean*
CONTROL	6.850 ± 0.105	6.876 ± 0.079	6.867 ± 0.081	6.865 ± 0.009
AUD	6.905 ± 0.125	6.829 ± 0.127	6.861 ± 0.110	6.864 ± 0.014
Mean	6.877 ± 0.117	6.853 ± 0.106	6.864 ± 0.095	


A)
Ethanol intake
(g/kg body weight/day)
8
6
4
2
0
0
6
12
18
24
30
36
42
Days
B)
Ethanol preference (%)
80
60
40
20
0
6
12
18
24
30
36
42
Days


A)
GLP-1R relative gene expression in PFC
(2-ΔΔCt)
B)
GLP-1R relative gene expression in NAc
(2-ΔΔCt)
C)
GLP-1R relative gene expression in HIP
(2-ΔΔCt)
D)
OPRM1 relative gene expression in NAc
(2-ΔΔCt)
E)
BDNF relative gene expression in HIP
(2-ΔΔCt)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
*
VEH
ETOH


A)
GLP-1R relative gene expression in PFC
(2-ΔΔCt)
B)
GLP-1R relative gene expression in NAc
(2-ΔΔCt)
C)
GLP-1R relative gene expression in HIP
(2-ΔΔCt)
D)
OPRM1 relative gene expression in NAc
(2-ΔΔCt)
E)
BDNF relative gene expression in HIP
(2-ΔΔCt)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
*
Controls
AUD