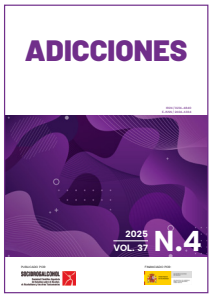
SOCIDROGALCOHOL
Sociedad Científica Española de Estudios sobre el Alcohol, el Alcoholismo y las otras Toxicomanías

ADICCIONES
2025 ■ VOL. 37 ■ N. 4 ■ PAGES 383–394
www.adicciones.es



ORIGINAL

Study of the Pleiotrophin/PTPRZ neurotrophic pathway in the hippocampus of rats exposed to chronic alcohol consumption and/or thiamine deficiency

Estudio de la ruta neurotrófica Pleiotrofina/PTPRZ en el hipocampo de ratas expuestas a consumo crónico de alcohol y/o deficiencia de tiamina

Rosario López-Rodríguez*,**,***; Marta Moya****; Esther Gramage*,**,***; Gonzalo Herradón+,*,**,***; Laura Orio+,***,****,*****.

*Department of Health and Pharmaceutical Sciences, School of Pharmacy, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain.

**Instituto de Estudios de las Adicciones, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain.

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

****Department of Psychobiology and Behavioral Science Methods, School of Psychology, Universidad Complutense de Madrid, Madrid, Spain.

*****Instituto de Investigación Sanitaria Hospital Universitario 12 de Octubre (imas12), Madrid, Spain.

+ Both authors contributed equally.

Abstract

Wernicke's encephalopathy (WE) is caused by thiamine deficiency (TD) whose main risk factor is alcohol use disorder. Pathogenic mechanisms associated with WE include mitochondrial dysfunction, oxidative stress and neuroinflammation. This study aims to explore the gene expression signature of certain candidate genes related to neuroinflammation, mitochondrial dysfunction and thiamine metabolism in the hippocampus from animals exposed to chronic alcohol consumption, thiamine deficiency or the combination of both.

Male Wistar rats (n=42) were randomly assigned to 4 experimental groups: control (C) receiving tap water or tap water plus thiamine (0.2 g/L), chronic alcohol (CA) forced ingestion for 36 weeks, TD diet and pyrithiamine for 12 days (TDD) and CA combined with TDD. The relative gene expression of neurotrophic factors (*Ptn, Mdk, Ptprz), proinflammatory molecules (Tlr4, Ccl2 and Hmgb1),* mitochondrial homeostatic factors *(Mfn1 and Mfn2) and thiamine metabolism (Tpk1)* was analyzed in RNA isolated from the hippocampus across all experimental groups. Differences in gene expression were assessed using non-parametric tests (Kruskal-Wallis).

Ptprz mRNA levels tended to be downregulated in the TDD group compared to controls (p=0.06, non-significant) and levels were significantly decreased related to the CA+TDD group (p<0.05). TDD group showed the lowest expression levels of *Ptn* across all experimental groups, and this decrease was statistically significant compared to the control and CA groups (p<0.05).

Our findings indicate a differential gene expression profile of the PTN-MDK-PTPRZ axis in the hippocampus of rats receiving a TD diet but not in the rest of the WE models analyzed (CA and CA+TDD).

Keywords: Wernicke, Korsakoff, thiamine deficiency, pleiotrophin, Protein Tyrosine Phosphatase Receptor Z, neuroinflammation, hippocampus

Resumen

La encefalopatía de Wernicke (WE) es una enfermedad neurológica causada por la deficiencia de tiamina (TD) cuyo principal factor de riesgo es el trastorno por uso del alcohol. El objetivo de este estudio es explorar el perfil de expresión de genes candidatos relacionados con neuroinflamación, disfunción mitocondrial y metabolismo de la tiamina en el hipocampo de animales expuestos a consumo crónico de alcohol (CA), una dieta deficiente en tiamina (TDD) o la combinación de ambos.

Se analizaron un total de 42 ratas Wistar macho incluidas en 4 grupos experimentales: control (C) que recibieron agua o agua suplementada con tiamina (0,2 g/L), alcohol crónico (CA) durante 36 semanas, dieta TD y piritiamina durante 12 días (TDD) y un grupo que combinaba CA+TDD. La expresión relativa de factores neurotróficos (*Ptn, Mdk, Ptprz), factores proinflamatorios (Tlr4, Ccl2 y Hmgb1), proteínas implicadas en homeostasis mitocondrial (Mfn1 y Mfn2) y enzimas del metabolismo de la tiamina (Tpk1)* se determinó a partir de ARNm obtenido del hipocampo de los distintos grupos experimentales. El análisis estadístico se realizó mediante el test no paramétrico Kruskal-Wallis.

La expresión de *Ptprz* tendía a ser menor en el grupo TDD comparado con el grupo C (no significativo) mientras que la disminución de *Ptprz* observada en el grupo TDD fue estadísticamente significativa cuando se comparaba con el grupo CA+TDD (p<0,05). Además, el grupo TDD mostró los menores niveles de expresión de *Ptn* y esta disminución fue estadísticamente significativa comparada con los grupos C y CA (p<0,05).

Nuestros resultados indican un perfil diferencial de expresión de la ruta PTN-MDK-PTPRZ en el hipocampo de ratas con una dieta TD distinto al observado en el resto de los modelos de encefalopatía WE analizados (CA y CA+TDD).

Palabras clave: Wernicke, Korsakoff, deficiencia de tiamina, pleiotrofina, receptor proteína tirosina Fosfatasa Z, neuroinflamación, hipocampo

■ *Received: July 2025; Accepted: November 2025.*

■ ISSN: 0214-4840 / E-ISSN: 2604-6334

■ **Send correspondence to:**

Laura Orio. Department of Psychobiology and Behavioral Science Methods, School of Psychology, Universidad Complutense de Madrid, Marid, Spain. Email: lorio@psi.ucm.es

Gonzalo Herradón. Department of Health and Pharmaceutical Sciences, School of Pharmacy, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain. Email: herradon@ceu.es

Thiamine is an essential vitamin (B1) acquired through the diet whose active form, thiamine pyrophosphate, is a required cofactor for crucial enzymes involved in energy metabolism (Jhala & Hazell, 2011; Zhao et al., 2014). Thiamine deficiency (TD) has been associated with mitochondrial dysfunction, increased oxidative and nitrosative stress and activation of inflammatory and cell damage processes (Moya et al., 2021; Moya et al., 2022b). Deregulation of those processes due to TD is associated with different neurological disorders (Abdou & Hazell, 2015; Cassiano et al., 2022; Jhala & Hazell, 2011; Martin et al., 2003; Moya et al., 2022a).

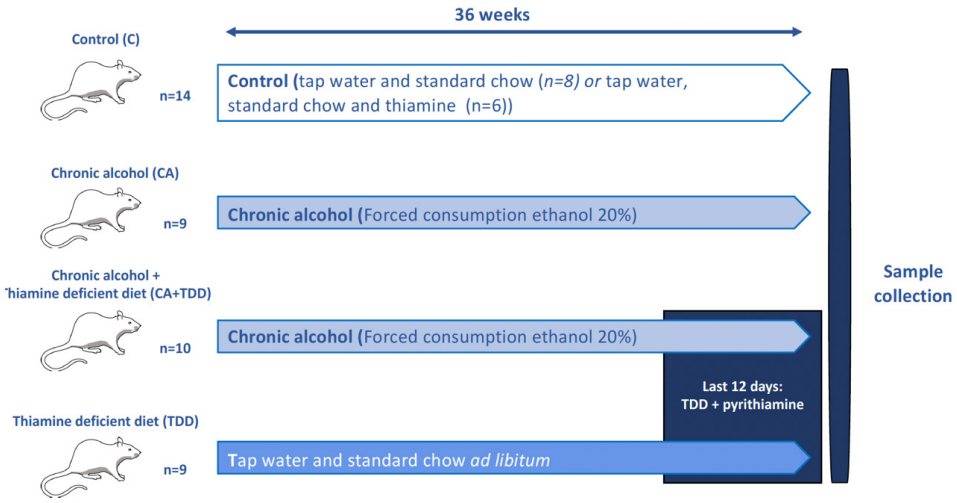
Wernicke's encephalopathy (WE) is an acute severe neurological condition caused by TD. The main cause of WE is alcohol use disorder (AUD), although WE has been also described in patients with non-alcohol related conditions such as inflammatory bowel disease, anorexia nervosa or patients after bariatric surgeries (Eva et al., 2023; Kohnke & Meek, 2021). The diagnosis of the disorder is frequently postmortem by brain imaging studies, with autopsy studies reporting a prevalence of WE between 0.4-2.8% and most of the cases associated with alcohol abuse (Abdou & Hazell, 2015; Li & Xing, 2025). Very few cases (20%) appear to be identified antemortem, and this misdiagnosis rate is alarmingly high for non-alcoholic WE. The disorder is diagnosed by a classic triad of symptoms (encephalopathy, ophthalmoplegia and ataraxia) but the three components are observed in a mere of 16% patients, the majority exhibiting only one or two components and some of them exhibiting a rare condition with no classic symptoms, especially in the early stages of non-alcoholic WE (Li & Xing, 2025). Other WE's main symptoms include cognitive (confusion, disinhibition) and motor (nystagmus, loss of balance, gait alterations, among others) disturbances (Kohnke & Meek, 2021; Oscar-Berman & Maleki, 2019). If left untreated, WE may progress to a more severe neurological condition called Korsakoff's syndrome (WKS), so it can be considered as a continuous, named Wernicke-Korsakoff's syndrome (Hammoud & Jimenez-Shahed, 2019), which is characterized by memory disorders (anterograde and retrograde amnesia) and psychiatric symptoms (confabulation and psychosis) (Arts et al., 2017; Kohnke & Meek, 2021; Oscar-Berman & Maleki, 2019). Neuroimaging studies in WE and WKS patients identified brain damage in different areas, being thalamus, mammillary bodies, hippocampus, frontal lobes or cerebellum, among the most affected areas (Jung et al., 2012). Currently, WE can be treated with thiamine supplementation to avoid progression of the disorder but there is no evidence of a beneficial pharmacological therapy to treat neurological damage in WKS (Arts et al., 2017; Sahu et al., 2025).

Modulation of the immune response in the central nervous system (CNS), especially the innate response mediated by glial cells, helps to restore and minimize the damage caused by pathogenic and toxic insults (Chew et al., 2006; Gomez-Nicola & Perry, 2015; Jung et al., 2019; Kielian, 2016; Lehnardt, 2010). However, chronic neuroinflammatory responses due to persistent insults or imbalance in homeostatic mechanisms contribute to a variety of neurological conditions (Gomez-Nicola & Perry, 2015; Jung et al., 2019; Kielian, 2016). Sustained neuroinflammation is also one of the proposed pathogenic mechanisms involved in WE (Abdou & Hazell, 2015; Cassiano et al., 2022; Moya et al., 2022a; Moya et al., 2022b; Moya et al., 2021; Toledo Nunes et al., 2019; Zahr et al., 2014). In this sense, an upregulation of main proinflammatory cytokines (such as IL1, IL6, TNFa or MCP1) and an increment of microglial activation markers were found in different brain areas (thalamus, inferior colliculus, or hippocampus, among others) in WE models (Toledo Nunes et al., 2019; Zahr et al., 2014). Interestingly, previous findings displayed an upregulation of the TLR4/MyD88 signaling pathway in the same WE model used in this study, specifically in the frontal cortex and cerebellum (Moya et al., 2022a; Moya et al., 2021). Pleiotrophin (PTN) and Midkine (MDK) are neurotrophic factors that act as regulators of neuroinflammation in various neurological conditions (Cañeque-Rufo et al., 2025; del Campo et al., 2021; Fernández-Calle et al., 2018, 2020; Rodríguez-Zapata et al., 2024; Vicente-Rodríguez et al., 2014; Vicente-Rodríguez et al., 2016). While the expression of PTN peaks at birth and readable levels are sustained in adulthood, MDK expression mainly occurs during embryonic development and can be induced in adults by different forms of tissue injury (Ross-Munro et al., 2020). PTN and MDK bind to different receptors, although Protein Tyrosine Phosphatase Receptor Z (PTPRZ, also known as RPTPβ/ζ) seems to be the most implicated in the regulation of neuroinflammation due to its main expression in the CNS (González-Castillo et al., 2015; Herradon et al., 2019; Ross-Munro et al., 2020). PTN and MDK inhibit the intrinsic tyrosine phosphatase activity of PTPRZ increasing the phosphorylation levels of its substrates such as β-catenin, Fyn or ALK (Herradon et al., 2019; Maeda et al., 1999). PTN and MDK have been found upregulated in diverse pathologies with a neuroinflammatory context such as Parkinson's disease, Alzheimer's disease, brain injury or after the administration of drugs of abuse (amphetamine or cocaine) (Herradon et al., 2019). In relation to alcohol consumption (main risk factor of WE and WKS), PTN was also upregulated after an acute ethanol administration in the mouse prefrontal cortex (Vicente-Rodríguez et al., 2014) and an increase of MK was also observed in the frontal cortex of AUD patients (Flatscher-Bader & Wilce, 2008).

Recent studies have revealed the important role of the PTN-RPTPZ pathway in hippocampal processes.

Figure 1

Experimental design and timeline of the treatments



Note. A total of 42 male Wistar rats were fed with standard chow and tap water ***ad libitum*** for 12 days prior to experimentation. After that, animals were randomly assigned to each experimental group: control [tap water and standard chow] (C, n=14[#]), chronic alcohol [20% w/v for 36 weeks] (CA, n=9), Thiamine deficiency [thiamine deficient diet and a daily pyrithiamine administration (0.25 mg/kg; i.p.) for the last 12 days] (TDD, n=9) and CA combined with TDD (CA+TDD, n=10). [#]Six control animals received oral thiamine (0.2 g/L) in the water. Experimental groups collected from (Moya, López-Valencia, et al., 2022). The rat image was obtained from BioArt Collection NIAID Visual & Medical Arts. 26/06/2025. Black Rat–Grey (BIOART-000054) NIAID BIOART Source: https://bioart.niaid.nih.gov/bioart/54 and its color was modified.

Interestingly, the loss of hippocampal neurogenesis induced by exposure to ethanol during adolescence is regulated by the administration of a selective RPTPZ inhibitor, MY10. Administering MY10 to mice completely prevented the loss of hippocampal neurogenesis caused by acute ethanol exposure during adolescence (Galán-Llario et al., 2023a). Additionally, previous studies have revealed evidence of sex-specific differences in the effects of chronic intermittent alcohol on glial responses and hippocampal neurogenesis (Galán-Llario et al., 2023b). Apart from alcohol-induced neuroinflammation, other studies suggest that endogenous PTN levels play an important role in regulating the acute systemic response to lipopolysaccharide (LPS) and the hippocampal microglial changes in young adult mice, as well as in the regulation of the long-term effects of LPS on the astrocytic response and neurogenesis in the hippocampus (Rodríguez-Zapata et al., 2024). However, the modulatory role of PTN-RPTPZ in hippocampal processes following chronic alcohol consumption and/or thiamine deficiency remains to be studied.

Therefore, this study aims to explore the gene expression signature of the PTN-MDK-PTPRZ axis and other candidate genes related to neuroinflammation, mitochondrial dysfunction and thiamine metabolism in the hippocampus from three different *in vivo* models that potentially may induce WE by chronic alcohol consumption (CA), TD diet and antagonism of thiamine (TDD) or the combination of CA and TDD (CA+TDD).

Materials and methods

Animals

Male Wistar rats (Envigo©, Barcelona, Spain) weighing 100–125 g at arrival were fed with standard food and tap water that were available *ad libitum* for 12 days prior to experimentation. After that (around PD 40), animals (n = 42) were randomly assigned to each experimental group. A detailed description of the animal housing can be found in a previous publication (Moya et al., 2021).

All procedures followed ARRIVAL guidelines and adhered to the guidelines of the Animal Welfare Committee of the Complutense University of Madrid (reference: PROEX 312-19) in compliance with Spanish Royal Decree 53/2013 and following European Directive 2010/63/EU on the protection of animals used for research and other scientific purposes.

Experimental groups

The experimental design is depicted in Figure 1. In this study, 4 experimental groups were employed: chronic alcohol (CA), TD diet + pyrithiamine (TDD), CA combined with TDD (CA+TDD) and control (C) group. A detailed description of the experimental groups can be found in a previous publication (Moya et al., 2022b).

CA group was exposed to forced consumption of an ethanol solution (limited access to a single bottle) based on the protocol described previously (Fernandez et al., 2016). The ethanol solution was prepared from ethanol 96° (Iberalcohol S.L., Madrid, Spain) in tap water. Alcohol was gradually introduced; started at 6% for 5 days, followed by another 5 days at 9%, 5 days at 12%, 2 days at 16% and finally

reaching 20%, which was maintained during the 36-week duration of the experiment. CA rats (n= 9) were provided with standard food *ad libitum* throughout the experiment.

In the TDD group (n= 9), animals were feed with the standard food and had access to a single bottle with tap water. In the last 12 days of the experiment, the chow was substituted by a TD diet (residual thiamine level <0.5 ppm; Teklad Custom Diet, Envigo, Madison, WI, USA), as well as a daily pyrithiamine hydrobromide administration (thiamine pyrophosphokinase inhibitor) (Sigma Aldrich, Madrid, Spain; 0.25 mg/kg; i.p.), as described previously (Moya et al., 2021).

In the CA+TDD group, animals received the same alcohol treatment as the CA group and in the last 12 days of the experiment the standard food was changed to the TD diet plus a daily pyrithiamine hydrobromide injection, as described for the TDD group (n= 10).

The C group had access to a single bottle with tap water and standard chow *ad libitum* for the entire duration of the study (n=8). An additional control group (n=6) supplemented with 0.2 g/L of thiamine in the water throughout the experiment (Moya et al., 2022b) was joined to this group since no significant changes were found in any parameters analyzed (C group, n=14).

During the last 12 days of protocol, C and CA groups received equivalent daily injections of saline (i.p.) to reproduce the same stress conditions in all animals.

Tissue Sample Collection

On day 12 of the TDD protocol, at least 1 h after treatment administration, the animals were decapitated after lethal injection of sodium pentobarbital (320 mg/kg, i.p., Dolethal®, Vétoquinol, Spain). The brains were immediately isolated from the skull, discarding the meninges and blood vessels. Samples of frontal cortex and cerebellum have been used and published in (Moya et al., 2022b). Hippocampus were also excised and frozen at −80 °C until assayed in this study.

Gene expression analysis

Total RNA from hippocampus (left hemisphere) was isolated using the Total RNA Isolation Kit (Nzytech, Lisbon, Portugal) according to manufacturer`s instructions. Then, 1,5 µg of RNA were reverse-transcribed to first-strand cDNA (First-strand cDNA Synthesis Kit, Nzytech, Lisbon, Portugal).

Quantitative real-time PCR was performed in duplicate for the relative quantification of *Ptprz, Ptn, Mdk, Tlr4, Ccl2, Hmgb1, Mfn1, Mfn2 and Tpk1* and by using the SsoAdvanced Universal SYBR Green Supermix kit (Bio-Rad, Hercules, CA, USA) in a CFX Opus Real-Time System (Bio-Rad, Hercules, CA, USA). The relative expression of each gene was calculated using *Rpl13* and *b2m* as reference genes, according to Livak method (Livak & Schmittgen, 2001). The primer sequences and experimental conditions are summarized in Supplementary Table 1.

Statistical analysis

Statistical analyses were performed using IBM-SPSS v28 software (IBM Corp., Armonk, N.Y., USA) and data were depicted using Graphpad Prism version 8 (San Diego, CA, United States). Data are presented as mean ± standard error of the mean (SEM). After assessing the non-normality of the data distribution (Kolmogorov-Smirnov test), differences in gene expression between experimental groups were analyzed using non-parametric tests (Kruskal-Wallis) and post-hoc comparisons were applied using the Bonferroni correction. A p value less than 0.05 was considered statistically significant.

Results

PTN-MDK-PTPRZ pathway

First, the relative gene expression of *Ptprz* and its ligands *Ptn* and *Mdk* was analyzed across all experimental groups (C; CA; TDD; CA+TDD) (Figure 2A-C). While the expression levels of *Ptprz* in the group exposed to chronic alcohol with or without TD diet (CA and CA+TDD) were similar to the control group, its gene expression tended to decrease in TDD group compared to controls (p=0.06, non-significant) and significantly decreased in TDD group compared to CA+TDD group (Figure 2A, p<0.05). Moreover, TDD group showed the lowest expression levels of *Ptn* across all experimental groups, and this decrease was statistically significant compared to the control and CA groups (Figure 2B, p<0.05). *Ptn* gene expression was also lower in the group receiving CA and TDD (CA+TDD) compared to the control and CA groups, although these differences did not reach statistical significance after Bonferroni correction. In contrast, no significant differences were observed in the expression of *Mdk* between the analyzed groups (Figure 2C).

Neuroinflammatory molecules

Expression levels of other genes associated with chronic inflammation (*Tlr4, Ccl2 and Hmgb1)* were also assessed in the hippocampus tissues of the different experimental groups (Figure 3). *Tlr4* gene expression was similar across all the experimental groups (Figure 3A). *Ccl2* expression appears to be slightly higher in those experimental groups receiving TDD (CA+TDD and TDD) compared to C and CA groups, despite being a non-significant tendency (Figure 3B). Regarding *Hmgb1,* its relative gene expression was almost stable across all experimental groups, independently of the treatment received (Figure 3C).

Mitochondrial homeostasis and thiamine metabolism

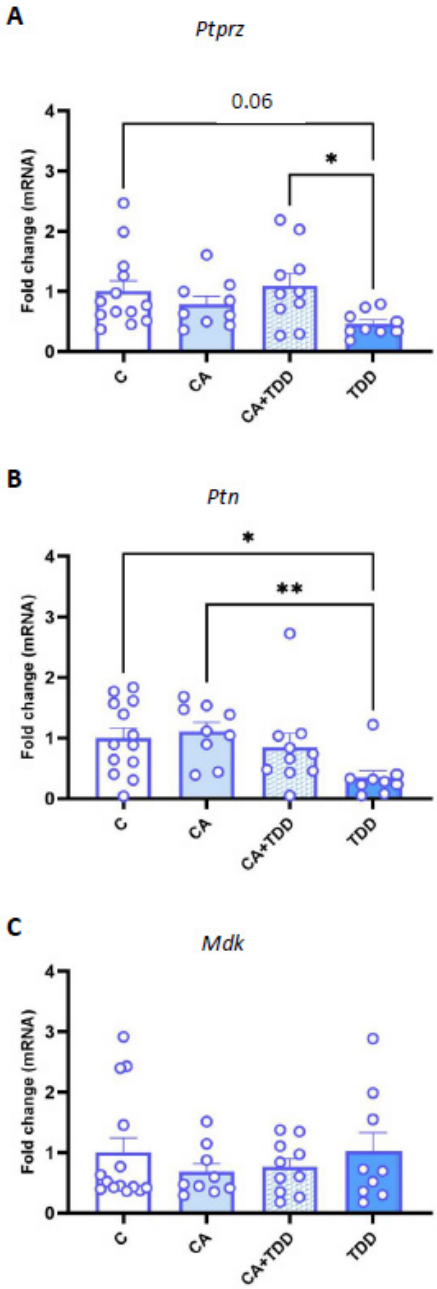
In addition, gene expression levels of *Mfn1* and *Mfn2*, crucial proteins implicated in mitochondrial health and homeostasis, were also analyzed (Figure 4). Interestingly,

Mfn1 expression tended to decrease in the TDD group (non-significant effect) while its expression in the rest of the experimental groups was similar (Figure 4A). Likewise, the lowest levels of *Mfn2* expression were found in the TDD group (non-significant effect), being its expression similar across the rest of the experimental groups (Figure 4B).

Finally, the gene expression levels of *Tpk1*, which encodes the enzyme involved in the conversion of thiamine to its

Figure 2

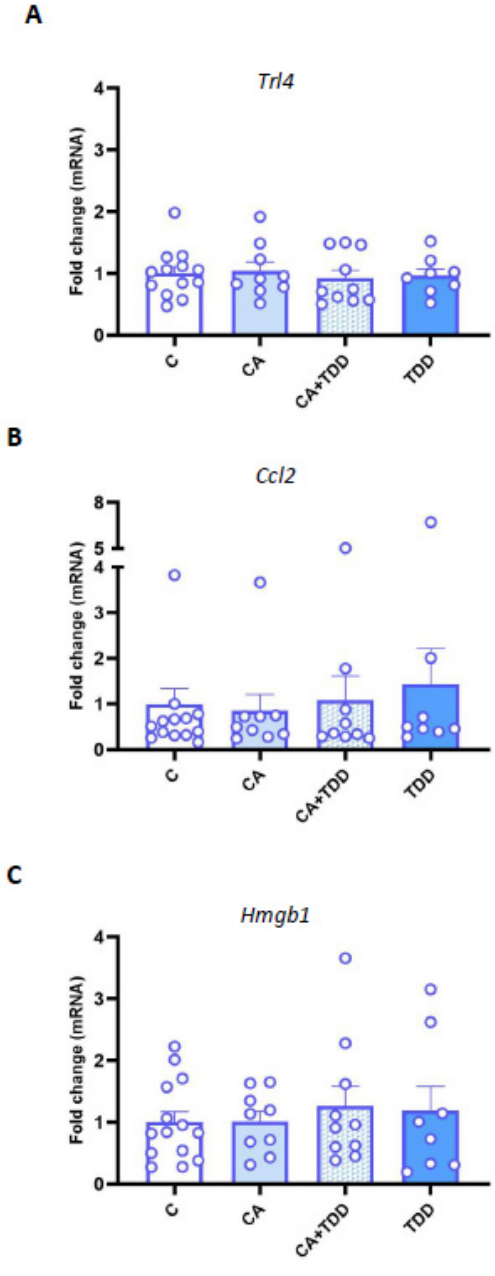
Relative gene expression of Ptprz and its ligands Ptn and Mdk across all experimental groups



Note. Graph represents data (mean ± S.E.M.) from the relative quantification of ***Ptprz*** (A), ***Ptn*** (B) and ***Mdk*** (C) mRNA levels in the hippocampus of male Wistar rats.
Control (C, n=14), chronic alcohol (CA, n=9), thiamine deficient diet and pyrithiamine for 12 days (TDD, n=9) and CA combined with TDD (CA+TDD, n=10). ***p* < 0.05 for significant differences among C ***vs.*** TDD and ****p* < 0.01 for significant differences among CA ***vs.*** TDD. No significant differences were observed in the expression of ***Mdk*** between the analyzed groups. Statistical significance was assessed by non-parametric tests (Kruskal-Wallis) and post-hoc comparisons (Bonferroni correction).

Figure 3

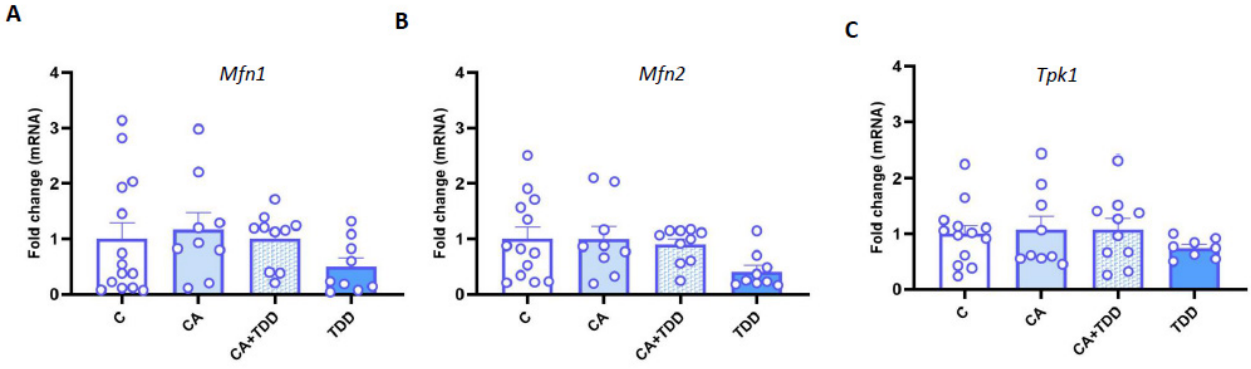
Relative expression of proinflammatory genes in the analyzed in vivo WE models



Note. Graph represents data (mean ± S.E.M.) from the relative quantification of ***Tlr4*** (A)***, Ccl2*** (B) ***and Hmgb1*** (C) mRNA levels in the hippocampus.
Control (C, n=14), chronic alcohol (CA, n=9), thiamine deficient diet and pyrithiamine for 12 days (TDD, n=9) and CA combined with TDD (CA+TDD, n=10). Statistical significance was assessed by non-parametric tests (Kruskal-Wallis) and post-hoc comparisons (Bonferroni correction). No significant differences were observed in the expression of ***Tlr4, Ccl2 and Hmgb1*** between the analyzed groups.

Figure 4

Expression levels of genes related to mitochondrial homeostasis and thiamine metabolism.



Note. Graph represents data (mean ± S.E.M.) from the relative quantification of ***Mfn1*** (A)***, Mfn2*** (B) ***and Tpk1*** (C).
Control (C, n=14), chronic alcohol (CA, n=9), thiamine deficient diet and pyrithiamine for 12 days (TDD, n=9) and CA combined with TDD (CA+TDD, n=10).
Statistical significance was assessed by non-parametric tests (Kruskal-Wallis) and post-hoc comparisons (Bonferroni correction). No significant differences were observed in the expression of ***Mfn1, Mfn2 and Tpk1*** between the analyzed groups.

active form, was measured (Figure 4C). Its expression levels did not differ between the experimental groups, although the lowest levels of *Tpk1* (non-significant) were found in the TDD group (Figure 4C).

Discussion

WE is a major neurological condition caused by TD, being AUD the main risk factor (Eva et al., 2023; Oscar-Berman & Maleki, 2019). Furthermore, WKS can be often developed in those WE patients that do not receive thiamine replacement therapy. Its severe effects, including amnesia and psychiatric disorders, impact significantly the quality of life of these patients (Kohnke & Meek, 2021; Oscar-Berman & Maleki, 2019). In this study, the gene expression signature of a set of candidate genes involved in neuroinflammation, mitochondrial dysfunction and thiamine metabolism was analyzed in three animal models potentially inducing WE (CA, CA+TDD and TDD) to characterize the contribution of alcohol and the nutritional deficiency of thiamine to this condition, specifically in the hippocampus. Our findings indicate a differential gene expression of the PTN-MDK-PTPRZ axis in the group receiving TD diet with no significant contribution of the alcohol groups (CA and CA+TDD).

PTPRZ is the main receptor for PTN and MDK at the CNS, where is found widely expressed. PTN and MDK are two neurotrophic factors involved in the regulation of neuroinflammatory mechanisms underlying different neurological conditions (Herradon et al., 2019). *Ptprz* was downregulated in the hippocampus of the TDD group; however, this decrease was not observed in the experimental groups exposed to chronic alcohol (36 weeks) without or with TD diet (CA and CA+TDD, respectively). In this sense, previous results from our group showed no changes in the expression of *Ptprz* in the mouse PFC after an acute exposure to ethanol (Rodríguez-Zapata et al., 2023). Therefore, our data suggest that *Ptprz* downregulation may be specific to TD diet and is not affected by chronic alcohol exposure as observed in CA and CA+TDD groups. However, further studies are needed to confirm that *Ptprz* expression is not modified by chronic alcohol consumption. Interestingly, *Ptprz* knockout mice exhibit altered social behavior and aggressivity suggestive of some positive symptoms of schizophrenia (also presented in WKS patients) as well as elevated levels of dopamine in PFC, amygdala and hippocampus (Cressant et al., 2017).

Strikingly, TD diet induced a significant decrease on *Ptn* expression while no significant differences were observed in *Mdk* across the groups analyzed. This is interesting because PTN and MDK are the only members of this family of cytokines, and highly overlap in structure and function (Herradon et al., 2005; Herradón & Pérez-García, 2014). However, the data presented here suggest a more prominent role of PTN in situations of TD. In addition, PTN and MDK have been found upregulated in diverse pathologies with a neuroinflammatory component (Herradon et al., 2019). In relation to alcohol consumption, *Ptn* expression was upregulated in the mouse PFC after an acute ethanol administration (Vicente-Rodríguez et al., 2014); however, after a 4-week adolescent intermittent access to ethanol model, we did not observe significant changes of *Ptn* expression in the mouse hippocampus (Galán-Llario et al., 2024). Furthermore, PTN seems to play a protective role against dopaminergic neural loss in different pathological contexts (Gombash et al., 2012; Gramage et al., 2010). In response to a toxic insult, such as amphetamine administrations, *Ptn* knockout mice displayed an intensified

dopaminergic neurotoxicity in the nigrostriatal pathway (Gramage et al., 2010), while *Ptn* overexpression showed protective neurotrophic effects in rodent models of Parkinson's disease (Gombash et al., 2012). Taking together the downregulation of *Ptn* and *Ptprz* in rats with TD diet, it is tempting to hypothesize that deficits in this neurotrophic signaling pathway could be involved in the severity of the brain injury caused by TD diet.

Neuroinflammation is one of the main pathogenic mechanisms underlying brain damage in WE (Cassiano et al., 2022; Eva et al., 2023; Toledo Nunes et al., 2019; Zahr et al., 2014; Zhao et al., 2014). Therefore, the gene expression pattern of *Tlr4, Ccl2 and Hmgb1* were assessed in the animal models of CA consumption and TD different WE models analyzed in the present study. Different studies support the role of the immune TLR4 response in the neuroinflammation observed in WE, particularly in the cortical and cerebellar areas (Moya et al., 2022a; Moya et al., 2021). For example, both TLR4 and HMGB1 protein levels were upregulated in the PFC after 12 days of TDD, whereas alterations of this pathway in the cerebellum were more evident after 16 days of TDD (Moya et al., 2021). In the current study, we did not find significant alterations in the expression of these proinflammatory molecules in the hippocampus after 12 days of TDD, suggesting that the peak of neuroinflammation in this structure may be happening at a different time-point, in accordance with the described different brain regional vulnerabilities to TD over time (Moya et al., 2021). Nevertheless, transcriptome analysis of an *ex vivo* TD model (organotypic hippocampal slice culture) did not show alterations in *Tlr4, Ccl2* or *Hmgb1* among 90 differentially expressed genes, including TNF and FoxO signaling pathways (Cassiano et al., 2022), in agreement with the data in the current study.

Additionally, the gene expression of *Mfn1* and *Mfn2* was determined. Regulation of mitochondrial dynamics is crucial for calcium and energy homeostasis in neurons (McCoy & Cookson, 2012; van Horssen et al., 2019), being MFN1 and MFN2 key for mitochondrial fusion (van Horssen et al., 2019; Wai & Langer, 2016). Both, *Mfn1* and *Mfn2*, tended to decrease in the TDD group (non-significant) while their expression in the rest of the experimental groups was very similar to the control group. Interestingly, *Mfn2* levels have been found to be decreased in the hippocampus of mice subjected to the drinking in the dark procedure (Mira et al., 2020). Furthermore, *Mfn2* inducible knockout mice displayed neurodegeneration through oxidative stress and neuroinflammation at hippocampus and cortex (Han et al., 2020). Therefore, further studies are needed to confirm a possible decrease in *Mfn2* expression in TD and its putative value as an early marker of mitochondrial dysfunction.

Finally, we analyzed *Tpk1,* a crucial protein in thiamine metabolism which encodes the enzyme involved in the conversion of thiamine to its active form (Jhala & Hazell, 2011; Zhao et al., 2014). Low *Tpk1* expression in the brain compared to other tissue seems to contribute to brain vulnerability to thiamine deficiency (Xia et al., 2024). In this sense, we did not find differences in the expression levels of Tpk1, suggesting that thiamine metabolism in the hippocampus may not be crucial in these WE models.

Taking all results together, this study shows a prominent role of the TDD model versus the chronic alcohol consumption model, since any alteration found in this study was present in the TDD experimental group. Similarly, other authors observed a pivotal role for TD in the expression of neuroinflammatory markers compared with models of chronic alcohol, where inflammatory markers displayed only minor modifications (Toledo Nunes et al., 2019). Similarly, regarding parameters of damage and behavioral correlates such as disinhibition, we previously observed that the changes in the CA+TDD group were highly dependent of the TDD (Moya et al., 2022b), confirming the stronger damage potential of this model.

Regarding behavioral correlates, the animals in the TDD group did not show a significant affectation of memory, although a trend in the Novel Object Recognition (NOR) test could be observed, since some TDD animals displayed higher latencies to explore the novel object and a mild decrease in the discrimination index (Moya et al., 2022b). In the present study, we also examined the potential correlation between two parameters of the NOR test (latency to novel object and discrimination index). Our analysis found no significant association between the levels of Ptn and_*Ptprz* expression and these NOR test results (data not shown). Thus, the significant decreases in *Ptn* and *Ptprz* expression observed in the hippocampus of the TDD group may be related to an initial step of hippocampus damage, in which memory and motor functions are not significantly compromised.

We are aware of some limitations of the study, as the inclusion of a combined control group (with and without thiamine supplementation) and the modest changes observed in the expression of certain candidate genes (*Ptn* and *Ptprz*) in the TDD group. In addition, in the animals of the current study we observed a trend toward declining the plasmatic thiamine levels in all CA animals (Moya et al., 2022b) although the active form (TDP) was not analyzed. In this regard, other authors have reported significant decreases in thiamine diphosphate (TDP) levels, the active form of thiamine, in red blood cells of animals that underwent similar CA and TDD protocols, as an objective measurement of thiamine deficiency (Toledo Nunes et al., 2019). Finally, we included only males in this study, which is an important limitation. To our knowledge, females have not been studied with these animal protocols. Considering the current gap in biomedical research regarding the effects on females and giving the growing body of

knowledge on sex differences in the biological impact of CA on brain parameters, including neuroinflammation, it is urgent to complete these investigations using females for comparative analyses. Despite these limitations, these preliminary findings regarding the PTN/PTPRZ axis may provide novel data on the initial steps of brain injury caused by a TD diet.

The data suggest that an overall deficit of the PTN/PTPRZ neurotrophic pathway, together with possible alterations in mitochondrial dynamics, may be events that precede the severe symptomatology of an advanced stage in WE. Further experiments are needed to confirm this hypothesis, which could lead to the development of new therapeutics to prevent neurological affectations and the progression of the disease. In this sense, it has been recently developed a BBB-permeable small molecule, called MY10, that mimics many of the PTN actions in the CNS, such as reducing alcohol consumption in different rodent models (Calleja-Conde et al., 2020; Fernández-Calle et al., 2018; Galán-Llario, Rodríguez-Zapata, Fontán-Baselga, et al., 2023).

Conclusion

Our findings indicate a differential gene expression profile of the PTN-MDK-PTPRZ axis in the hippocampus of rats receiving a thiamine deficient diet and pyrithiamine for 12 days but not in the rest of the WE models analyzed (CA and CA+TDD). The data suggest that deficits of the PTN/PTPRZ neurotrophic pathway may precede the more severe and advanced stages of WE. Further studies elucidating the roles of PTN and its receptor in WE may open new therapeutic avenues to prevent the development and progression of WKS.

Acknowledgments

This work was supported by National Plan on Drug abuse, Ministerio de Sanidad of Spain (grants 2023I018 to G.H. and 2024I044 to. E.G.), by Ministerio de Ciencia e Innovación (Spain), cofounded by FEDER (European Union) (grant number PID2021-127256OB-I00 to L.O.) and by ISCIII Redes de Investigación Cooperativa Orientadas a Resultados en Salud (RICORS), Red de Investigación en Atención Primaria de Adicciones (RIAPAd; grant RD24/0003/0011 to G.H. and RD24/0003/0010 to L.O.).

Conflicts of interest

The authors declare no conflict of interest.

References

Abdou, E. & Hazell, A. S. (2015). Thiamine Deficiency: An Update of Pathophysiologic Mechanisms and Future Therapeutic Considerations. *Neurochemical Research*, *40*(2), 353–361. https://doi.org/10.1007/s11064-014-1430-z

Arts, N., Walvoort, S. & Kessels, R. (2017). Korsakoff's syndrome: A critical review. *Neuropsychiatric Disease and Treatment*, *Volume 13*, 2875–2890. https://doi.org/10.2147/NDT.S130078

Calleja-Conde, J., Fernández-Calle, R., Zapico, J. M., Ramos, A., de Pascual-Teresa, B., Bühler, K., Echeverry-Alzate, V., Giné, E., Rodríguez de Fonseca, F., López-Moreno, J. A. & Herradón, G. (2020). Inhibition of Receptor Protein Tyrosine Phosphatase β/ζ Reduces Alcohol Intake in Rats. *Alcoholism: Clinical and Experimental Research*, *44*(5), 1037–1045. https://doi.org/10.1111/acer.14321

Cañeque-Rufo, H., Fontán-Baselga, T., Galán-Llario, M., Zuccaro, A., Sánchez-Alonso, M. G., Gramage, E., Ramos-Álvarez, M. del P. & Herradón, G. (2025). Pleiotrophin deletion prevents high-fat diet-induced cognitive impairment, glial responses, and alterations of the perineuronal nets in the hippocampus. *Neurobiology of Disease*, *205*, 106776. https://doi.org/10.1016/j.nbd.2024.106776

Cassiano, L. M. G., Oliveira, M. S., Pioline, J., Salim, A. C. M. & Coimbra, R. S. (2022). Neuroinflammation regulates the balance between hippocampal neuron death and neurogenesis in an ex vivo model of thiamine deficiency. *Journal of Neuroinflammation*, *19*(1), 272. https://doi.org/10.1186/s12974-022-02624-6

Chew, L., Takanohashi, A. & Bell, M. (2006). Microglia and inflammation: Impact on developmental brain injuries. *Mental Retardation and Developmental Disabilities Research Reviews*, *12*(2), 105–112. https://doi.org/10.1002/mrdd.20102

Cressant, A., Dubreuil, V., Kong, J., Kranz, T. M., Lazarini, F., Launay, J.-M., Callebert, J., Sap, J., Malaspina, D., Granon, S. & Harroch, S. (2017). Loss-of-function of PTPR γ and ζ, observed in sporadic schizophrenia, causes brain region-specific deregulation of monoamine levels and altered behavior in mice. *Psychopharmacology*, *234*(4), 575–587. https://doi.org/10.1007/s00213-016-4490-8

del Campo, M., Fernández-Calle, R., Vicente-Rodríguez, M., Martín Martínez, S., Gramage, E., Zapico, J. M., Haro, M. & Herradon, G. (2021). Role of Receptor Protein Tyrosine Phosphatase β/ζ in Neuron–Microglia Communication in a Cellular Model of Parkinson's Disease. *International Journal of Molecular Sciences*, *22*(13), 6646. https://doi.org/10.3390/ijms22136646

Eva, L., Brehar, F.-M., Florian, I.-A., Covache-Busuioc, R.-A., Costin, H. P., Dumitrascu, D.-I., Bratu, B.-G., Gla-

van, L.-A. & Ciurea, A. V. (2023). Neuropsychiatric and Neuropsychological Aspects of Alcohol-Related Cognitive Disorders: An In-Depth Review of Wernicke's Encephalopathy and Korsakoff's Syndrome. *Journal of Clinical Medicine*, *12*(18), 6101. https://doi.org/10.3390/jcm12186101

Fernandez, G. M., Stewart, W. N. & Savage, L. M. (2016). Chronic Drinking During Adolescence Predisposes the Adult Rat for Continued Heavy Drinking: Neurotrophin and Behavioral Adaptation after Long-Term, Continuous Ethanol Exposure. *PLOS ONE*, *11*(3), e0149987. https://doi.org/10.1371/journal.pone.0149987

Fernández-Calle, R., Galán-Llario, M., Gramage, E., Zapatería, B., Vicente-Rodríguez, M., Zapico, J. M., de Pascual-Teresa, B., Ramos, A., Ramos-Álvarez, M. P., Uribarri, M., Ferrer-Alcón, M. & Herradón, G. (2020). Role of RPTPβ/ζ in neuroinflammation and microglia-neuron communication. *Scientific Reports*, *10*(1), 20259. https://doi.org/10.1038/s41598-020-76415-5

Fernández-Calle, R., Vicente-Rodríguez, M., Pastor, M., Gramage, E., Di Geronimo, B., Zapico, J. M., Coderch, C., Pérez-García, C., Lasek, A. W., de Pascual-Teresa, B., Ramos, A. & Herradón, G. (2018). Pharmacological inhibition of Receptor Protein Tyrosine Phosphatase β/ζ (PTPRZ1) modulates behavioral responses to ethanol. *Neuropharmacology*, *137*, 86–95. https://doi.org/10.1016/j.neuropharm.2018.04.027

Flatscher-Bader, T. & Wilce, P. A. (2008). Impact of Alcohol Abuse on Protein Expression of Midkine and Excitatory Amino Acid Transporter 1 in the Human Prefrontal Cortex. *Alcoholism: Clinical and Experimental Research*, *32*(10), 1849–1858. https://doi.org/10.1111/j.1530-0277.2008.00754.x

Galán-Llario, M., Rodríguez-Zapata, M., Fontán-Baselga, T., Cañeque-Rufo, H., García-Guerra, A., Fernández, B., Gramage, E. & Herradón, G. (2024). Pleiotrophin Overexpression Reduces Adolescent Ethanol Consumption and Modulates Ethanol-Induced Glial Responses and Changes in the Perineuronal Nets in the Mouse Hippocampus. *CNS Neuroscience & Therapeutics*, *30*(12). https://doi.org/10.1111/cns.70159

Galán-Llario, M., Rodríguez-Zapata, M., Fontán-Baselga, T., Gramage, E., Vicente-Rodríguez, M., Zapico, J. M., de Pascual-Teresa, B., Lasek, A. W. & Herradón, G. (2023a). Inhibition of RPTPβ/ζ reduces chronic ethanol intake in adolescent mice and modulates ethanol effects on hippocampal neurogenesis and glial responses in a sex-dependent manner. Neuropharmacology, 227, 109438. https://doi.org/10.1016/j.neuropharm.2023.109438

Galán-Llario, M., Rodríguez-Zapata, M., Gramage, E., Vicente-Rodríguez, M., Fontán-Baselga, T., Ovejero-Benito, M.C., Pérez-García C, Carrasco J., Moreno-Herradón M, Sevillano J., Ramos-Álvarez P., Zapico, J. M., de Pascual-Teresa, B., Ramos A. & Herradón, G. (2023b). Receptor protein tyrosine phosphatase β/ζ regulates loss of neurogenesis in the mouse hippocampus following adolescent acute ethanol exposure. Neurotoxicology, 94:98-107. https://doi.org/10.1016/j.neuro.2022.11.008

Gombash, S. E., Lipton, J. W., Collier, T. J., Madhavan, L., Steece-Collier, K., Cole-Strauss, A., Terpstra, B. T., Spieles-Engemann, A. L., Daley, B. F., Wohlgenant, S. L., Thompson, V. B., Manfredsson, F. P., Mandel, R. J. & Sortwell, C. E. (2012). Striatal Pleiotrophin Overexpression Provides Functional and Morphological Neuroprotection in the 6-Hydroxydopamine Model. *Molecular Therapy*, *20*(3), 544–554. https://doi.org/10.1038/mt.2011.216

Gomez-Nicola, D. & Perry, V. H. (2015). Microglial Dynamics and Role in the Healthy and Diseased Brain. *The Neuroscientist*, *21*(2), 169–184. https://doi.org/10.1177/1073858414530512

GonzÃ¡lez-Castillo, C., OrtuÃ±o-SahagÃºn, D., GuzmÃ¡n-Brambila, C., PallÃ s, M. & Rojas-MayorquÃn, A. E. (2015). Pleiotrophin as a central nervous system neuromodulator, evidences from the hippocampus. *Frontiers in Cellular Neuroscience*, *8*. https://doi.org/10.3389/fncel.2014.00443

Gramage, E., Rossi, L., Granado, N., Moratalla, R. & Herradón, G. (2010). Genetic inactivation of Pleiotrophin triggers amphetamine-induced cell loss in the substantia nigra and enhances amphetamine neurotoxicity in the striatum. *Neuroscience*, *170*(1), 308–316. https://doi.org/10.1016/j.neuroscience.2010.06.078

Hammoud, N. & Jimenez-Shahed, J. (2019). Chronic Neurologic Effects of Alcohol. *Clinics in Liver Disease*, *23*(1), 141–155. https://doi.org/10.1016/j.cld.2018.09.010

Han, S., Nandy, P., Austria, Q., Siedlak, S. L., Torres, S., Fujioka, H., Wang, W. & Zhu, X. (2020). Mfn2 Ablation in the Adult Mouse Hippocampus and Cortex Causes Neuronal Death. *Cells*, *9*(1), 116. https://doi.org/10.3390/cells9010116

Herradon, G., Ezquerra, L., Nguyen, T., Silos-Santiago, I. & Deuel, T. F. (2005). Midkine regulates pleiotrophin organ-specific gene expression: Evidence for transcriptional regulation and functional redundancy within the pleiotrophin/midkine developmental gene family. *Biochemical and Biophysical Research Communications*, *333*(3), 714–721. https://doi.org/10.1016/j.bbrc.2005.05.160

Herradón, G. & Pérez-García, C. (2014). Targeting midkine and pleiotrophin signalling pathways in addiction and neurodegenerative disorders: Recent progress and perspectives. *British Journal of Pharmacology*, *171*(4), 837–848. https://doi.org/10.1111/bph.12312

Herradon, G., Ramos-Alvarez, M. P. & Gramage, E. (2019). Connecting Metainflammation and Neuroinflammation Through the PTN-MK-RPTPβ/ζ Axis: Relevance in

Therapeutic Development. *Frontiers in Pharmacology*, *10*. https://doi.org/10.3389/fphar.2019.00377

Jhala, S. S. & Hazell, A. S. (2011). Modeling neurodegenerative disease pathophysiology in thiamine deficiency: Consequences of impaired oxidative metabolism. *Neurochemistry International*, *58*(3), 248–260. https://doi.org/10.1016/j.neuint.2010.11.019

Jung, Y. J., Tweedie, D., Scerba, M. T. & Greig, N. H. (2019). Neuroinflammation as a Factor of Neurodegenerative Disease: Thalidomide Analogs as Treatments. *Frontiers in Cell and Developmental Biology*, *7*. https://doi.org/10.3389/fcell.2019.00313

Jung, Y.-C., Chanraud, S. & Sullivan, E. V. (2012). Neuroimaging of Wernicke's Encephalopathy and Korsakoff's Syndrome. *Neuropsychology Review*, *22*(2), 170–180. https://doi.org/10.1007/s11065-012-9203-4

Kielian, T. (2016). Multifaceted roles of neuroinflammation: the need to consider both sides of the coin. *Journal of Neurochemistry*, *136*(S1), 5–9. https://doi.org/10.1111/jnc.13530

Kohnke, S. & Meek, C. L. (2021). Don't seek, don't find: The diagnostic challenge of Wernicke's encephalopathy. *Annals of Clinical Biochemistry: International Journal of Laboratory Medicine*, *58*(1), 38–46. https://doi.org/10.1177/0004563220939604

Lehnardt, S. (2010). Innate immunity and neuroinflammation in the CNS: The role of microglia in Toll-like receptor-mediated neuronal injury. *Glia*, *58*(3), 253–263. https://doi.org/10.1002/glia.20928

Li, S. & Xing, C. (2025). Wernicke encephalopathy: A mini review of the clinical spectrum, atypical manifestations, and diagnostic challenges. *Frontiers in Neurology*, *16*. https://doi.org/10.3389/fneur.2025.1566366

Livak, K. J. & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Maeda, N., Ichihara-Tanaka, K., Kimura, T., Kadomatsu, K., Muramatsu, T. & Noda, M. (1999). A Receptor-like Protein-tyrosine Phosphatase PTPζ/RPTPβ Binds a Heparin-binding Growth Factor Midkine. *Journal of Biological Chemistry*, *274*(18), 12474–12479. https://doi.org/10.1074/jbc.274.18.12474

Martin, P. R., Singleton, C. K. & Hiller-Sturmhöfel, S. (2003). The role of thiamine deficiency in alcoholic brain disease. *Alcohol Research & Health : The Journal of the National Institute on Alcohol Abuse and Alcoholism*, *27*(2), 134–142.

McCoy, M. K. & Cookson, M. R. (2012). Mitochondrial quality control and dynamics in Parkinson's disease. *Antioxidants & Redox Signaling*, *16*(9), 869–882. https://doi.org/10.1089/ARS.2011.4019

Mira, R. G., Lira, M., Quintanilla, R. A. & Cerpa, W. (2020). Alcohol consumption during adolescence alters the hippocampal response to traumatic brain injury. *Biochemical and Biophysical Research Communications*, *528*(3), 514–519. https://doi.org/10.1016/j.bbrc.2020.05.160

Moya, M., Escudero, B., Gómez-Blázquez, E., Rebolledo-Poves, A. B., López-Gallardo, M., Guerrero, C., Marco, E. M. & Orio, L. (2022a). Upregulation of TLR4/MyD88 pathway in alcohol-induced Wernicke's encephalopathy: Findings in preclinical models and in a postmortem human case. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/fphar.2022.866574

Moya, M., López-Valencia, L., García-Bueno, B. & Orio, L. (2022b). Disinhibition-Like Behavior Correlates with Frontal Cortex Damage in an Animal Model of Chronic Alcohol Consumption and Thiamine Deficiency. *Biomedicines*, *10*(2), 260. https://doi.org/10.3390/biomedicines10020260

Moya, M., San Felipe, D., Ballesta, A., Alén, F., Rodríguez de Fonseca, F., García-Bueno, B., Marco, E. M. & Orio, L. (2021). Cerebellar and cortical TLR4 activation and behavioral impairments in Wernicke-Korsakoff Syndrome: Pharmacological effects of oleoylethanolamide. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *108*, 110190. https://doi.org/10.1016/j.pnpbp.2020.110190

Oscar-Berman, M. & Maleki, N. (2019). Alcohol Dementia, Wernicke's Encephalopathy, and Korsakoff's Syndrome. In M. L. Alosco & R. A. Stern (Eds.), *The Oxford Handbook of Adult Cognitive Disorders* (pp. 742–758). Oxford University Press. https://doi.org/10.1093/oxfordhb/9780190664121.013.33

Rodríguez-Zapata, M., Galán-Llario, M., Cañeque-Rufo, H., Sevillano, J., Sánchez-Alonso, M. G., Zapico, J. M., Ferrer-Alcón, M., Uribarri, M., Pascual-Teresa, B. de, Ramos-Álvarez, M. del P., Herradón, G., Pérez-García, C. & Gramage, E. (2023). Implication of the PTN/RPTPβ/ζ Signaling Pathway in Acute Ethanol Neuroinflammation in Both Sexes: A Comparative Study with LPS. *Biomedicines*, *11*(5), 1318. https://doi.org/10.3390/biomedicines11051318

Rodríguez-Zapata, M., López-Rodríguez, R., Ramos-Álvarez, M. del P., Herradón, G., Pérez-García, C. & Gramage, E. (2024). Pleiotrophin modulates acute and long-term LPS-induced neuroinflammatory responses and hippocampal neurogenesis. *Toxicology*, *509*, 153947. https://doi.org/10.1016/j.tox.2024.153947

Ross-Munro, E., Kwa, F., Kreiner, J., Khore, M., Miller, S. L., Tolcos, M., Fleiss, B. & Walker, D. W. (2020). Midkine: The Who, What, Where, and When of a Promising Neurotrophic Therapy for Perinatal Brain Injury. *Frontiers in Neurology*, *11*. https://doi.org/10.3389/fneur.2020.568814

Sahu, P., Verma, H. K. & Bhaskar, L. (2025). Alcohol and alcoholism associated neurological disorders: Current updates in a global perspective and recent recommen-

dations. *World Journal of Experimental Medicine*, *15*(1). https://doi.org/10.5493/wjem.v15.i1.100402

Toledo Nunes, P., Vedder, L. C., Deak, T. & Savage, L. M. (2019). A Pivotal Role for Thiamine Deficiency in the Expression of Neuroinflammation Markers in Models of Alcohol-Related Brain Damage. *Alcoholism: Clinical and Experimental Research*, *43*(3), 425–438. https://doi.org/10.1111/acer.13946

van Horssen, J., van Schaik, P. & Witte, M. (2019). Inflammation and mitochondrial dysfunction: A vicious circle in neurodegenerative disorders? *Neuroscience Letters*, *710*. https://doi.org/10.1016/J.NEULET.2017.06.050

Vicente-Rodríguez, M., Pérez-García, C., Ferrer-Alcón, M., Uribarri, M., Sánchez-Alonso, M. G., Ramos, M. P. & Herradón, G. (2014). Pleiotrophin differentially regulates the rewarding and sedative effects of ethanol. *Journal of Neurochemistry*, *131*(5), 688–695. https://doi.org/10.1111/jnc.12841

Vicente-Rodríguez, M., Rojo Gonzalez, L., Gramage, E., Fernández-Calle, R., Chen, Y., Pérez-García, C., Ferrer-Alcón, M., Uribarri, M., Bailey, A. & Herradón, G. (2016). Pleiotrophin overexpression regulates amphetamine-induced reward and striatal dopaminergic denervation without changing the expression of dopamine D1 and D2 receptors: Implications for neuroinflammation. *European Neuropsychopharmacology*, *26*(11), 1794–1805. https://doi.org/10.1016/j.euroneuro.2016.09.002

Wai, T. & Langer, T. (2016). Mitochondrial Dynamics and Metabolic Regulation. *Trends in Endocrinology and Metabolism: TEM*, *27*(2), 105–117. https://doi.org/10.1016/J.TEM.2015.12.001

Xia, Y., Qian, T., Fei, G., Cheng, X., Zhao, L., Sang, S. & Zhong, C. (2024). Low expression of thiamine pyrophosphokinase-1 contributes to brain susceptibility to thiamine deficiency. *NeuroReport*, *35*(15), 1000–1009. https://doi.org/10.1097/WNR.0000000000002094

Zahr, N. M., Alt, C., Mayer, D., Rohlfing, T., Manning-Bog, A., Luong, R., Sullivan, E. V. & Pfefferbaum, A. (2014). Associations between in vivo neuroimaging and postmortem brain cytokine markers in a rodent model of Wernicke's encephalopathy. *Experimental Neurology*, *261*, 109–119. https://doi.org/10.1016/j.expneurol.2014.06.015

Zhao, Y., Wu, Y., Hu, H., Cai, J., Ning, M., Ni, X. & Zhong, C. (2014). Downregulation of transketolase activity is related to inhibition of hippocampal progenitor cell proliferation induced by thiamine deficiency. *BioMed Research International*, *2014*, 572915. https://doi.org/10.1155/2014/572915

Supplementary Information

Supplementary Table 1.

List of primers used for gene expression analysis by quantitative real-time PCR.

Gene	Primer Forward/Reverse	T_a (ºC)
Ptn	5′-TTGGGGAGAATGTGACCTCAATAC-3′ 5′-TCTCCTGTTTCTTGCCTTCCTTT-3′	60
Ptprz1	5′-ACCACCAACACCCATCTTTCC-3′ 5′-CAGCTCTGCACTTCCTGGTAAA-3′	60
Mdk	5′-CCCGTGAGCGAGATGCAG-3′ 5′-CAGGTCCACTCCGAACACTC-3′	60
Hmgb1	5′-TACAGAGCGGAGAGAGTGAGG-3′ 5′-GACATTTTGCCTCTCGGCTT-3′	60
Mfn1	5′-CTGGGACGGAATGAGTGACC-3′ 5′-CATGTGAGGGGCCCAATCTT-3′	60
Mfn2	5′-AGAGGCGATTTGAGGAGTGC-3′ 5′-CGCTCTTCCCGCATTTCAAG-3′	60
Tpk1	5′-CCCGCTATGGAGCATGTCTT-3′ 5′-GCTTTTCTCCAAAGATGCCGA-3′	60
Ccl2	5′-AGATCTGTGCTGACCCCAAT-3′ 5′-GGTGCTGAAGTCCTTAGGGT-3′	60
Tlr4	5′-GATCTGAGCTTCAACCCCCTG-3′ 5′-GTACCAAGGTTGAGAGCTGGT-3′	60
Rpl13	5′-GAGGCGAAACAAATCCACGG-3′ 5′-GTTAGCTGCGTGGCCAATTT-3′	60
b2m	5′-GAGCCCAAAACCGTCACCT-3′ 5′-GAAGATGGTGTGCTCATTGC-3′	60

Note. Ptn, Pleiotrophin***; Ptprz1,*** Protein tyrosine phosphatase receptor type Z1***; Mdk,*** Midkine***; Hmgb1,*** High mobility group box 1***; Mfn1,*** Mitofusin 1***; Mfn2,*** Mitofusin 2***; Tpk1,*** Thiamin pyrophosphokinase 1***; Ccl2***, C-C motif chemokine ligand 2; ***Tlr4,*** Toll-like receptor 4; ***Rpl13,*** Ribosomal protein L13; and ***b2m***, Beta 2 macroglobulin. Ta, annealing temperature.
Primers were designed by using the online tool Primer-BLAST, NIH (https://www.ncbi.nlm.nih.gov/tools/primer-blast).