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SECRETARÍA DE ESTADO
DE SERVICIOS SOCIALES

DELEGACIÓN DEL GOBIERNO
PARA EL PLAN NACIONAL SOBRE DROGAS

2019

Boletín de producción científica relacionada con las convocatorias de ayudas a proyectos de investigación

Boletín 2/2019

INTRODUCCIÓN

En este boletín número 2 de 2019, se presenta la producción científica que ha dado lugar la convocatoria de ayudas a la investigación de la DGPNSD, del año 2015. Esta convocatoria financia proyectos sobre adicciones de investigación básica, clínica y sociosanitaria, y en general, estos proyectos tienen una duración de tres anualidades. Por ello, ahora se presentan los artículos publicados relacionados con los proyectos subvencionados en 2015.

Por último, agradecer el esfuerzo realizado por todos y todas las que han hecho posible este boletín especialmente, los equipos y los centros de investigación.

SUMARIO

En este número:

ALCOHOL

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PPARα KNOCKOUT MICE SHOW A COMPENSATORY EXPRESSION OF THE PPARγ ISOFORM PRIMARLY UNDER ETHANOL STIMULATORY CONDITIONS.

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INTRODUCTION

PPARα potential target for treatment of neuroinflammation-associated neuropsychiatric diseases

Endogenous PPARα agonists → Acylethanolamides → ↓ neuroinflammation through TLR-4 pathway

EtOH binge drinking → Frontal cortex neuroinflammation

AIMS OF THE STUDY

1. To characterize the neuroinflammatory profile of KO mice under physiological conditions.

2. To analyze the effects of EtOh binge challenge in neuroinflammatory markers alterations induced by lack of PPARα.

METHODS

1. C57BL/6J Wt and PPARα KO mice (n=6) → RIP → Frontal Cortex Samples → rt-PCR analysis

2. C57BL/6J Wt and PPARα KO mice (n=6-8) → 4 days DID protocol → RIP → Frontal Cortex samples → rt-PCR analysis; Plasma samples → ELISA kit

Drinking in the dark (DID) protocol (Rhodes et al., 2015)

Day 1, 2, 3: 8AM Lights off → 3 hours → H_2O → 2 hours → EtOH groups: EtOH 20% (v/v) Control groups: H_2O

Day 4: 8AM Lights off → 3 hours → H_2O → 4 hours → EtOH groups: EtOH 20% (v/v) Control groups: H_2O → 1,5 hours → Sacrifice

RESULTS

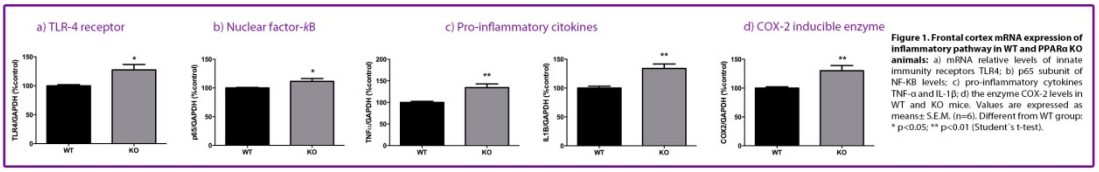

Figure 1. Frontal cortex mRNA expression of inflammatory pathway in WT and PPARα KO animals: a) mRNA relative levels of innate immunity receptors TLR4; b) p65 subunit of NF-KB levels; c) pro-inflammatory cytokines TNF-α and IL-1β; d) the enzyme COX-2 levels in WT and KO mice. Values are expressed as means± S.E.M. (n=6). Different from WT group: * p<0.05; ** p<0.01 (Student´s t-test).

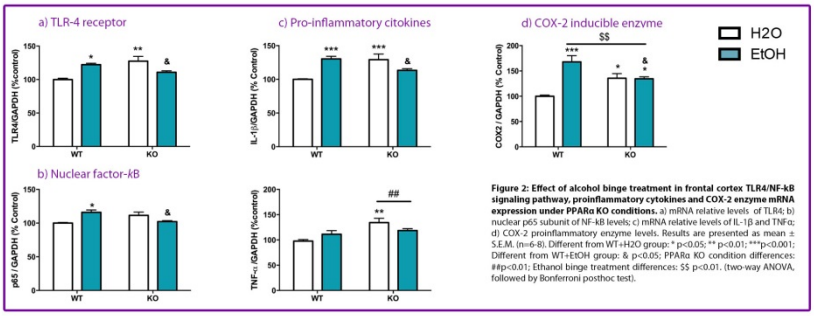

Figure 2: Effect of alcohol binge treatment in frontal cortex TLR4/NF-kB signaling pathway, proinflammatory cytokines and COX-2 enzyme mRNA expression under PPARα KO conditions. a) mRNA relative levels of TLR4; b) nuclear p65 subunit of NF-kB levels; c) mRNA relative levels of IL-1β and TNFα; d) COX-2 proinflammatory enzyme levels. Results are presented as mean ± S.E.M. (n=6-8). Different from WT+H2O group: * p<0.05; ** p<0.01; ***p<0.001; Different from WT+EtOH group: & p<0.05; PPARα KO condition differences: ##p<0.01; Ethanol binge treatment differences: $$ p<0.01. (two-way ANOVA, followed by Bonferroni posthoc test).

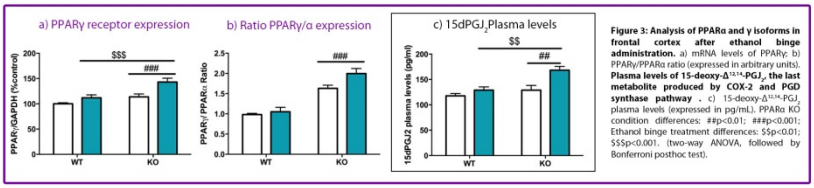

Figure 3: Analysis of PPARα and γ isoforms in frontal cortex after ethanol binge administration. a) mRNA levels of PPARγ; b) PPARγ/PPARα ratio (expressed in arbitrary units). **Plasma levels of 15-deoxy-$\Delta^{12,14}$-PGJ_2, the last metabolite produced by COX-2 and PGD synthase pathway** . c) 15-deoxy-$\Delta^{12,14}$-PGJ_2 plasma levels (expressed in pg/mL). PPARα KO condition differences: ##p<0.01; ###p<0.001; Ethanol binge treatment differences: $$p<0.01; $$$p<0.001. (two-way ANOVA, followed by Bonferroni posthoc test).

SUMMARY OF RESULTS

Results indicated that PPARα KO mice showed higher inflammatory state under physiological conditions. We verified an up-regulation of TLR4/NF-κB pathway expression and also an increase in several pro-inflammatory mediators in frontal cortex.

After Drinking in the Dark ethanol administration we observed an increase in main inflammatory markers in the WT ethanol group as expected. However, ethanol animals lacking PPARα receptors showed lower levels of TLR4, p65, COX2 and IL-1β mRNA levels compared with ethanol WT group.

Additionally, PPARα KO animals showed a compensatory upregulation of the PPARγ isoform, which is also anti-inflammatory, primarily under ethanol stimulating conditions. Indeed, PPARγ/α ratio in KO animals was bigger under ethanol challenge. Furthermore, plasma levels of 15d-PGJ2, an endogenous PPARγ ligand, were similarly upregulated in KO animals, especially in ethanol-treated animals.

CONCLUSIONS

These results highlight an anti-inflammatory homeostatic role of PPARα in physiological conditions and indicate that the lack of PPARα may induce a compensatory PPARγ isoform up-regulation mainly after inflammatory stimulus such as ethanol binge exposure.

ACKNOWLEDGEMENTS

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MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD

SECRETARÍA DE ESTADO DE SERVICIOS SOCIALES E IGUALDAD

DELEGACIÓN DEL GOBIERNO PARA EL PLAN NACIONAL SOBRE DROGAS

YOUNG ALCOHOL BINGE DRINKERS SHOW IMMUNE/INFLAMMATORY ALTERATIONS WITH HIGER SUSCEPTIBILITY IN WOMEN: correlations with neuropsychological abilities.

Orio L[1,2], Antón M[1], Rodríguez-Rojo IC[3], Correas A[3], García-Bueno B[4], Corral M[6], Rodríguez de Fonseca F[1,2,5], García-Moreno LM[1], Maestú F[3], Cadaveira F[6]

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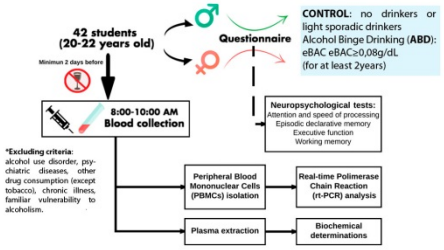
INTRODUCTION

Alcohol binge drinking is a pattern of heavy alcohol consumption increasingly used by adolescents and young adults. Preclinical evidence indicates that alcohol binge induces peripheral inflammation and an exacerbated neuroimmune response that may participate in the alcohol-induced cognitive/behavioral dysfunctions.

AIMS OF THE STUDY

1. To characterize the presence of blood inflammatory markers in 20 years old university students, identified as binge drinkers for at least 2 years.
2. To study sex differences in the inflammatory/immune response to alcohol binge in young drinkers.
3. To investigate possible correlations with a batery of neuropsychological tests assesing cognitive and executive functioning.

METHODS



RESULTS

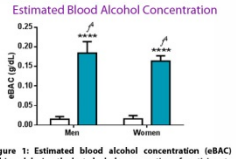


Figure 1: Estimated blood alcohol concentration (eBAC) achieved during the last alcohol consumption of participants in the study. The eBAC was calculated by algorithm based on the information of the last alcohol consumption within the last month. Results are presented as mean ± S.E.M. Different from control group: **** p<0.0001 (two-way ANOVA) or ƒ4p<0.0001 (Student´s t-test).

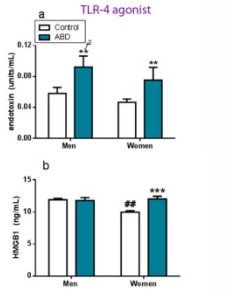


Figure 2: Levels of blood endotoxin (lipopolysaccharide, LPS) and High Mobility Group Box 1 (HMGB1) in young men and women alcohol drinkers. a) Levels of endotoxin in control and ABD subjects; b) Differences in plasma HMGB1 levels were detected between young men and women. Results are presented as mean ± S.E.M. Different from control group: **p<0.01 (two-way ANOVA treatment overall effect) or ƒ2p<0.01 (Student´s t-test). Analysis of the interaction (treatment x sex) (b): ***p<0.001 different from control; comparison between men and women in the same group: ##p<0.01 (Bonferroni´s multiple comparison post hoc test).

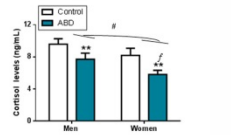


Figure 6: Plasma cortisol levels in alcohol binge drinkers. Results are presented as mean ± S.E.M. Different from control group: **p<0.01 (two-way ANOVA) or ƒp<0.05 (Student´s t-test). Overall effect of sex: #p<0.05 (two-way ANOVA).

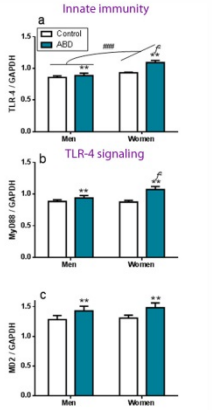


Figure 3: Up-regulation of innate immune receptor TLR4 and associated signaling molecules in blood of alcohol binge drinkers. a) TLR4 mRNA levels; b) TLR4 adaptor protein MyD88 (needed for intracellular signaling cascade) mRNA levels; c) TLR4 co-receptor MD2 mRNA levels. Results are presented as mean ± S.E.M. Different from control group: **p<0.01 (two-way ANOVA) or ƒ2p<0.01 (Student´s t-test); sex differences: ##p<0.001 (two-way ANOVA).

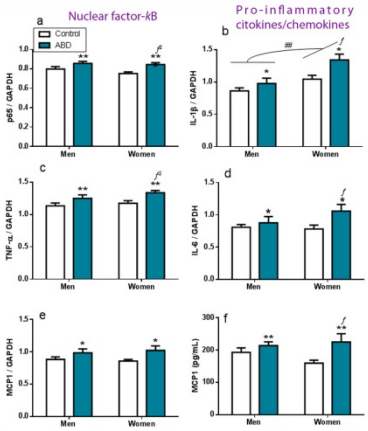


Figure 4: Induction of NF-kB p65 subunit, proinflammatory cytokines (TNF-α, IL-1β and IL-6), and the chemokine MCP-1 in peripheral blood mononuclear cells (PBMC) of alcohol binge drinkers. a) p65 subunit of NF-kB mRNA levels; b) mRNA levels of IL-1β; c) mRNA levels of TNF-α; d) mRNA levels of IL-6; e) mRNA levels of the chemokine MCP-1; f) plasma protein levels of MCP-1. Results are presented as mean ± S.E.M. Different from control group in the same condition: *p<0.05;**p<0.01 (two-way ANOVA) or ƒp<0.05 (Student´s t-test). Overall effect of sex: ##p<0.01 (two-way ANOVA).

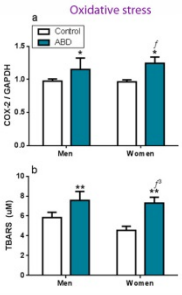


Figure 5: Levels of the enzyme COX-2 and lipid peroxidation status in alcohol binge drinkers. a) COX-2 mRNA levels; b) Levels of lipid peroxides measured by thiobarbituric acid reactive substances (TBARS) method. Results are presented as mean ± S.E.M. Different from control group in the same condition: *p<0.05;**p<0.01 (two-way ANOVA) or ƒp<0.05 ; ƒ3p<0.001 (Student´s t-test).

Table 1. Correlations between eBAC achieved during last alcohol consumption and immune/inflammatory activation in subjects of the study. r = Pearson/Spearman coefficient of correlation. p value (2-tailed) indicates statistical significance:(*):≤0.05; (**):<0.01. (§) Indicates positive correlation found only in women (r= 0.517; p value= 0.016*).

Immune/ Inflammatory marker	eBAC	
	Correlation Coefficient	
	r	p value
LPS	0.320	0.050 (*)
HMGB-1 (§)	0.202	0.206
TLR-4	0.477	0.005 (**)
MyD88	0.478	0.002 (**)
MD-2	0.271	0.104
TNF-α	0.474	0.002 (**)
IL-1β	0.323	0.071
IL-6	0.268	0.099
p65	0.365	0.037 (*)
MCP-1	0.315	0.048 (*)
Cox-2	0.365	0.029 (*)
TBARS	0.370	0.017 (*)
Cortisol	-0.360	0.024 (*)

Table 2. Correlations found between immune/inflammatory markers and neuropsychological tests in women drinkers. The table shows a summary of the significant correlations found between z-scores of different neuropsychological tests and biochemical markers related with inflammation in women drinkers. Low punctuation in tests where we found negative correlations indicate worse performance, whereas high scores in The Five Digit Test indicate poor performance, and a positive correlation was found in this case. r = Pearson coefficient of correlation; p value: *p<0.05 and **p<0.001.

Neuropsychological Test		Inflammatory marker	Correlation Coefficient r	p-value
Executive Function				
Five Digit Test	Flexibility	HMGB-1	0.620	0.032*
		MCP-1	0.699	0.025*
	Inhibition	MCP-1	0.938	0.002**
Rey-Osterrieth Complex Figure	Copying	COX-2	-0.716	0.009**
Iowa Gambling Task	Total score	EBAC	-0.645	0.032*
Episodic declarative memory				
Logical Memory	Immediate recall	TLR-4	-0.620	0.031*
		MCP-1	-0.709	0.015*
		IL-6	-0.601	0.039*
	Delayed recall	LPS	-0.634	0.049*

Young alcohol binge drinkers (ABDs) showed increased plasma endotoxin levels, innate immune over-activation and peripheral inflammation compared with controls, together with decreased cortisol levels. Women ABDs (with equivalent levels of intoxication than men) showed higher TLR4-mediated inflammatory response and elevated HMGB1 levels. Additionally, higher levels of LPS or inflammatory markers such as HMGB1, TLR4, MCP-1, IL-6, or COX-2 correlated with worse episodic memory and executive functioning in women ABDs but not men.

CONCLUSIONS

These results emphasize possible risky consequences of alcohol use in binge episodes during the young period, and call attention to sex differences in the alcohol-induced immune/inflammatory and neurocognitive response.

ACKNOWLEDGEMENTS

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MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD
SECRETARÍA DE ESTADO DE SERVICIOS SOCIALES E IGUALDAD
DELEGACIÓN DEL GOBIERNO PARA EL PLAN NACIONAL SOBRE DROGAS

ALCOHOL BINGE EPISODES DISRUPT PROTEINS CONFORMING THE INTESTINAL AND BLOOD-BRAIN BARRIERS. STUDY OF THE PROTECTIVE EFFECTS OF OLEOYLETHANOLAMIDE.

Orio L[1], Antón M[1], Ballesta A[1], Alen F[1], García Y, Caso JR[2], de Fonseca FR[1], García-Bueno B[2], Rodríguez-González A[1]

[1] Department of Psychobiology and Methods in Behavioral Science and [2] Department of Pharmacology and Toxicology, Universidad Complutense de Madrid, Spain. **Abstract control number : 3589** (Session number:761)

BACKGROUND & PURPOSE: Alcohol binge drinking induces peripheral inflammation that may affect the brain promoting neuroinflammation and cognitive decline(1,2). Pharmacological pretreatment with the biolipid oleoylethanolamide (OEA), which is part of the acylethanolamide family, has shown to reduce both peripheral inflammation and neuroinflammation in rodents(2). In this study we tested whether the alcohol-induced peripheral inflammation and neuroinflammation are related with disruptions in the intestinal barrier and in the blood-brain barrier using an animal model of alcohol binge drinking, and the possible protective effects of OEA.

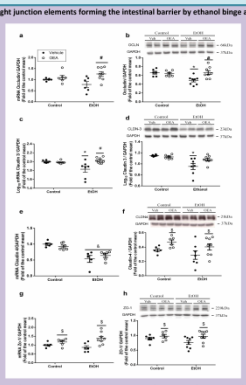
Hour	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
7 a.m		5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%
3 p.m	10mg/kg OEA i.p OR 20mg/kg OEA i.g 5g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	9 a.m sacrifice
11 p.m	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	5mg/kg OEA i.p OR 20mg/kg OEA i.g 3g/kg alcohol 30%	

Bacterial translocation studies in MLNs, blood, spleen and liver

qPCR & Western blot analysis in prefrontal cortex and colon

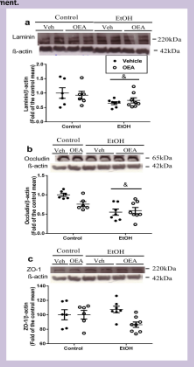
EXPERIMENTAL APPROACH: Adults male Wistar rats weighting 200 g were exposed to alcohol binge episodes by intragastric administrations of ¯3g/kg of alcohol every 8h during 4 consecutive days. Samples of brain frontal cortex and colonic intestinal tissue were collected to measure the integrity of proteins conforming the intestinal and blood-brain barriers, and plasma, spleen, liver and mesenteric lymph nodes (MLN) were collected in sterile conditions for determination of bacterial load. Data were analyzed by 2-way ANOVA comparing the factors alcohol/water oral administration versus OEA/vehicle i.p. treatment, followed by Bonferroni *post hoc* test when appropriate.

Alterations in colonic tight junction elements forming the intestinal barrier by ethanol binge administration and OEA i.p. pre-treatment.



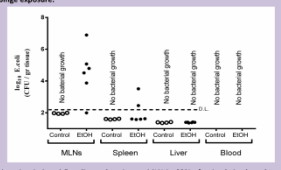
Colonic mRNA relative levels (left panels) and western blot analyses (right panels) of occludin (a, b), claudin-3 (c, d), claudin-4 (e, f) and zonula occludens-1 (ZO-1) (g, h). Results are presented as biological samples with the mean ± S.E.M. Overall ethanol effect: &p<0.05 (two-way ANOVA). Overall OEA effect: $p<0.05 (two-way ANOVA). Different from control group in the same condition: *p<0.05; differences between ethanol and ethanol+OEA groups: #p<0.05 (two-way ANOVA, Bonferroni post hoc test after interaction).

Alterations in the tight junction elements forming blood-brain barrier by ethanol binge administration and OEA i.p. pre-treatment.



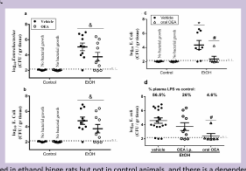
Western blot analyses of laminin (a), occludin (b) and zonula occludens-1 (ZO-1) (c) in the prefrontal cortex. Results are presented as biological with the mean ± S.E.M. Overall ethanol effect: &p<0.05 (two-way ANOVA).

Bacteria colony-forming units (CFUs) of Escherichia coli (E. coli) in mesenteric lymph nodes (MLN), spleen, liver and blood after ethanol binge exposure.



Ethanol binge administration induced E. coli translocation to MLN in 83% of animals in the ethanol group and to the spleen in 33% of animals. Animals with no bacterial growth are represented under the detection limit (D.L., discontinued horizontal line). Data are corrected by tissue weight (except blood samples) and expressed as log 10.

Enterobacteriaceae (a) and E. coli (b) translocation to mesenteric lymph nodes (MLN) in rats exposed to alcohol binge protocol and pharmacological OEA i.p. pre-treatment, and effects of oral OEA administration in ethanol-induced bacterial and LPS translocation in MLNs.



Presence of bacteria was detected in ethanol binge rats but not in control animals, and there is a dependence between these variables (Chi square 15.39, &p<0.05). There were no significant differences between OEA- or vehicle pretreated animals within the ethanol groups (Student's t test,p>0.05, n.s.) (c) oral OEA pre-treatment in ethanol-induced bacterial translocation in MLN. Data are expressed as biological samples with mean ± S.E.M. d) comparison between i.p. and oral OEA pre-treatments in bacterial translocation and in percentage of LPS increase in plasma (upper panel). Data are expressed as biological replicates and the mean ± S.E.M. Different from control group:*p<0.05. Different from EtOH+vehicle: #p<0.05 (Mann Whitney (c), Kruskal-Wallis, Dunn's post-hoc test (d)).

CONCLUSION: These results suggest that the anti-inflammatory actions of OEA may be due in part by a local action of this biolipid in the intestine, protecting from the alcohol binge-induced tight junction protein disruption and highlight a role of OEA in the regulation of the gut-brain axis altered by alcohol abuse.

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1.Silberman J.A., White A.M., Swartzwelder H.S. & Crews F.T. (2002) Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats. *Pharmacol Biochem Behav* 72: 521-532.
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Characterization of the Brain Innate Immune System in an animal model of Wernicke-Korsakoff Syndrome

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Abstract control number: 3620

INTRODUCTION: Wernicke-Korsakoff Syndrome (WKS) is the consequence of a severe deficiency of thiamin (B1 vitamin) that is typically related to alcohol abuse. The clinical course of WKS begins with Wernicke's Encephalopathy (W), characterized by ataxia and cognitive dysfunction, and may continue with the Korsakoff Syndrome (WK), characterized by irreversible brain damage. The molecular mechanism responsible of the transition between both stages is unknown, although some evidences suggest a role for neuroinflammation and oxidative/nitrosative stress.

OBJETIVE: To characterize an animal model of both pathological states, investigating also alterations on innate immune *Toll-like receptor 4* and tested a potential approach to manage such alterations based on the systemic administration of the anti-inflammatory molecule oleylethanolamide (OEA, endogenous lipid mediator of the acylethanolamide s family).

Phase I. Characterization of the rat model of Wernicke Encephalopathy and Wernicke-Korsakoff Syndrome

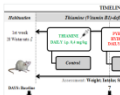


FIGURE 1 | Experimental schedule indicating the model generated by combining a feeding of a thiamine free diet with the administration (i.p. injection) of a thiamine antagonist, such as pyrithiamine (W state). A glucose load was given to accelerate neurodegeneration and the progression from W to WKS.

Onset and progression of neurological/behavioral symptoms and signs

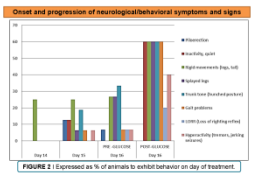


FIGURE 2 | Expressed as % of animals to exhibit behavior on day of treatment.

W animals showed a loss of body weight starting from day 12, reaching around the 10% of the body weight loss at the end of the experiment

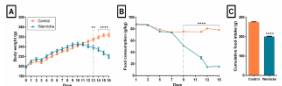
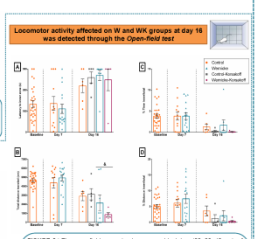


FIGURE 3 | **Effect of thiamine deprivation on body weight and food consumption.** Data are presented as mean SEM. **(A)** Pattern of body change weight in the 2 groups (Control (n=12) Wernicke (n=16), one-way ANOVA, significantly different from Control beginning on day 13 of treatment; **(B)** Food consumption (g/kg) decreased significantly beginning on day 9 of treatment; **(C)** Cumulative food intake. Student's T test: **p< 0.05; **** p<0.0001.

Locomotor activity affected on W and WK groups at day 16 was detected through the *Open-field test*



Impaired performance was exhibited by W and WK animals in the *Rotarod test* at day 16

FIGURE 4 | **(A)** Latency to fall, the amount of time that the rats remained on the rod during 10 min at constant velocity (15 rpm), and **(B)** Maximum Velocity reached, where the rod was initially rotated at a speed of 4 rpm after which it was accelerated gradually to 40 rpm over the course of 30 secs. Both of them were recorded as indicators of their motor coordination, before of the treatment (Baseline) and at days 7 and 16. Data are presented as mean SEM. Student's T test on day 7 (C (n=12), W (n=16). Two-way ANOVA on day 16 (C (n=6), CK (n=6), W (n=8), WK (n=8). Overall Wernicke effect: &p<0.05; $&p<0.01

FIGURE 5 | The open-field apparatus is a square black box (80 80 42 cm), of which the base was divided into 6 6 cm equal squares with legible white lines. On the software screen, the platform surface was divided into two regions as the centre (inner zone) and the edge. Each rat was placed in the corner of the floor and then allowed to enjoy independent movement and explore freely for 5 minutes. Behaviour evaluation parameters **(A)** Latency to inner zone (s), **(B)** Total distance traveled (cm), **(C)** % Time inner/total, **(D)** % Distance inner/total were measured before of the treatment (Baseline) and at days 7 and 16. Data are presented as mean SEM. Student's T test on day 7 (C (n=12), W (n=16); Two-way ANOVA on day 16 (C (n=6), CK (n=6), W (n=8), WK (n=8). Overall Wernicke effect: &p<0.05.

Phase II. To test the anti-inflammatory/neuroprotective properties of the satiety factor OEA in our rat model of Wernicke Encephalopathy and Wernicke-Korsakoff Syndrome

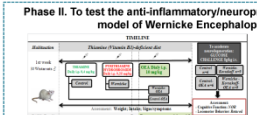


FIGURE 6 | Experimental design of the study, with 12 days of thiamine deprivation treatment to be able to evaluate the cognitive function before the motor function is totally affected. The systemic administration of the anti-inflammatory OEA began on day 7 until the last day.

OEA treatment suggests a prevention of the impaired motor coordination

FIGURE 7 | *Rotarod test* before of onset of the treatment (Baseline) and at days 8 and 12. Data are presented as mean SEM. Although the effect did not reach significance, there is a trend to WK-OEA group improves the **(A)** Latency to fall and **(B)** Maximum Velocity endured.

Novel Object Recognition test (NOT) showed a mild cognitive impairment in W and WK animals in recognition memory

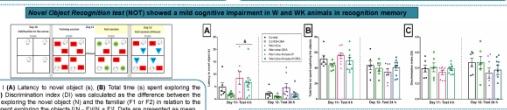


FIGURE 8 | **(A)** Latency to novel object (s), **(B)** Total time (s) spent exploring the objects, **(C)** Discrimination index (DI) was calculated as the difference between the time spent exploring the novel object (N) and the familiar (F1 or F2) in relation to the total time spent exploring the objects ([N - F)/(N + F)]. Data are presented as mean SEM. During the training phase, no differences in total exploration times were observed (data not shown). Two-way ANOVA; (C (n=6), C-CEA (n=6); W: to WK (n=8); W-OEA to WK-OEA (n=9). Overall Wernicke effect: &p<0.05.

Plasma parameters measured after pharmacological treatments

OEA blocked the elevation of the HMGB1/TLR4/MyD88 signaling pathway and increased the levels of anti-inflammatory cytokine IL-10 in frontal cortex

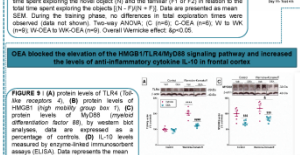


FIGURE 9 | **(A)** protein levels of TLR4 (Toll-like receptors 4), **(B)** protein levels of HMGB1 (high mobility group box 1), **(C)** protein levels of MyD88 (myeloid differentiation factor 88), by western blot analyses, data are expressed as a percentage of controls. **(D)** IL-10 levels measured by enzyme-linked immunosorbent assays (ELISA). Data represents the mean S.E.M. (n=6 per group). Two-way ANOVA, Bonferroni's post-hoc test: Overall Wernicke-Korsakoff effect: &p<0.05; overall OEA effect $$$p<0.001. Interaction different from control group: ***p<0.0001. Different from Wernicke-Korsakoff vehicles: #p<0.05, ### p<0.0001.

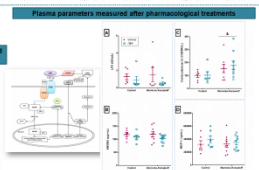


FIGURE 10 | **(A)** LPS (bacterial lipopolysaccharides, endotoxin gut permeability indicator), **(B)** HMGB1 danger signal, **(C)** corticosterone and **(D)** MCP-1 (monocyte chemoattractant protein-1), levels detected by Enzyme-linked immunosorbent assay (ELISA). Data represents the mean S.E.M. (n=6-9). Two-way ANOVA; Overall Wernicke-Korsakoff effect &p<0.05. Although not significative, results suggest an increase of the TLR4 ligand lipopolysaccharide levels in the WK animals, which could be inhibited by OEA.

CONCLUSIONS: Our results indicate the pharmacological modulation of innate immunity through OEA could be an attractive therapeutical strategy to manage the transition between reversible to irreversible structural and functional damage in the Wernicke-Korsakoff Syndrome.

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EVALUACIÓN DE LOS EFECTOS DEL CANNABIDIOL EN UN NUEVO MODELO CRÓNICO DE TRASTORNO DE ESTRÉS POST-TRAUMÁTICO

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INTRODUCCIÓN Y OBJETIVOS

El Trastorno de Estrés Post-traumático (TEPT) es una patología psiquiátrica heterogénea y de difícil manejo terapéutico (1,2). El desarrollo de nuevos modelos animales que reflejen mejor las alteraciones conductuales y neurológicas producidas en esta patología es imprescindible para evaluar nuevas estrategias farmacoterapéuticas (3).

En los últimos años, numerosos estudios avalan el uso del cannabidiol (CBD) para el abordaje de distintos trastornos de ansiedad (4), y recientemente se ha revisado su potencial utilidad para el manejo terapéutico del TEPT (5).

Los objetivos de este estudio son:
1) Validar y caracterizar un nuevo modelo crónico de TEPT.
2) Evaluar los efectos del CBD sobre la respuesta emocional (condicionamiento al miedo) de ratones expuestos al modelo de TEPT.

MATERIAL Y MÉTODOS

Animales
Se emplearon ratones machos de la cepa C57BL/6J de 3 semanas de edad (Charles River, Lille, Francia) estabulados en condiciones ambientales controladas (temperatura 23 ± 2ºC; ciclo luz-oscuridad 12 h:12 h). Todos los experimentos fueron aprobados por el Órgano Evaluador de Proyectos de la Universidad Miguel Hernández, siguiendo las consideraciones que establece el Real Decreto 53/2013.

Fármaco
El CBD (STI Pharmaceuticals, Essex, Reino Unido) se administró por vía intraperitoneal (i.p.) disuelto en su correspondiente vehículo (VEH; etanol:cremophor:salino; proporción 1:1:18), utilizando las dosis de 5 y 15 mg/kg seleccionadas conforme a datos preliminares y la literatura disponible.

Modelo crónico de TEPT
Los ratones fueron sometidos durante 5 semanas a los estímulos estresantes que se indican a continuación, siguiendo el diagrama temporal recogido en la Figura 1:
- Aplicación de descarga eléctrica: los ratones fueron expuestos a una descarga eléctrica de 1,0 mA durante 10 s.
- Exposición a la orina de un depredador natural (zorro): los ratones fueron introducidos durante 15 minutos en una jaula en cuya zona central se colocó un tubo falcon con orificios que contenía una gasa impregnada con 4 ml de orina de zorro.
- Restricción de movimiento: los ratones fueron introducidos de forma individual en un dispositivo de retención durante un período de 15 minutos.
- Jaula inclinada: durante su ciclo de oscuridad y durante un período de 12-14 horas, las jaulas se colocaron con una inclinación de 30º.
- Lecho húmedo: durante su ciclo de oscuridad y durante un período de 12-14 horas, los ratones fueron expuestos a jaulas con serrín húmedo.
- Deprivación de comida: durante su ciclo de oscuridad y durante un período de 12-14 horas, los ratones no tuvieron acceso a su pienso (agua *ad libitum*).

Condicionamiento al miedo
Se evaluó la memoria condicionada al estímulo aversivo (descarga eléctrica) exponiendo a los ratones al mismo contexto donde previamente habían recibido la descarga eléctrica (sin re-exposición a la misma), midiendo el tiempo de "congelación", evidenciado por la completa inmovilidad del animal (salvo para realizar los movimientos necesarios de respiración).

DETALLE TEMPORAL DEL PROCEDIMIENTO EXPERIMENTAL - MODELO DE TEPT EN RATONES C57BL/6J

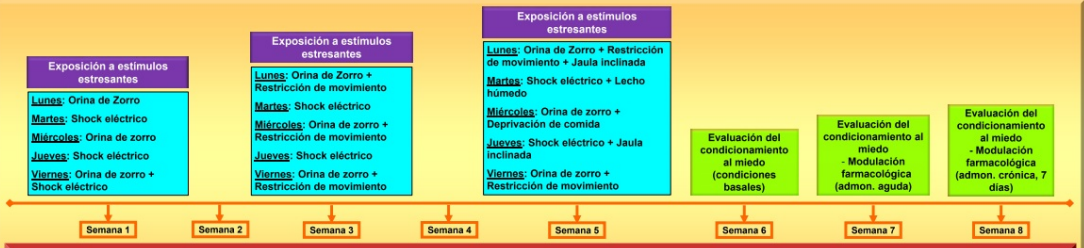

Figura 1. Diagrama temporal con el detalle de las diferentes fases de inducción del modelo de TEPT en ratones C57BL/6J. Se aplicaron los estímulos estresantes en semanas alternas durante un período total de 5 semanas. Posteriormente, se evaluó la respuesta emocional de los ratones mediante el paradigma de condicionamiento al miedo a nivel basal (semana 6), y el efecto de la modulación farmacológica con CBD administrado de forma aguda (semanas 7) y crónica (semana 8).

RESULTADOS - EVALUACIÓN DEL CONDICIONAMIENTO AL MIEDO

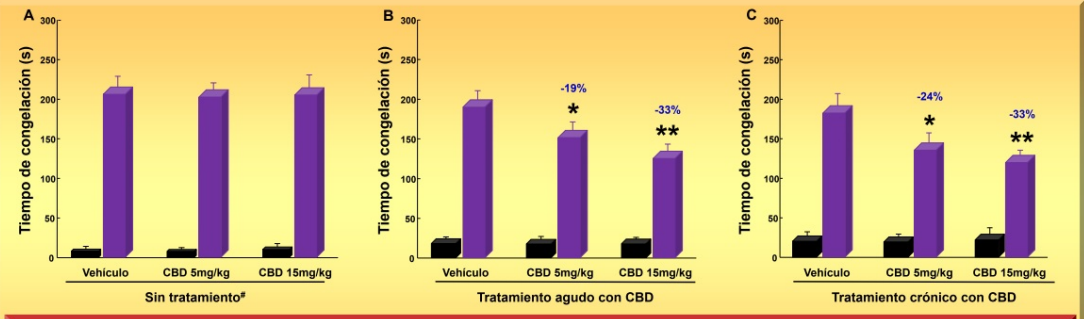

Figura 2. Evaluación de la conducta de condicionamiento al miedo mediante la re-exposición al contexto donde los ratones C57BL/6J recibieron la descarga eléctrica. A) Evaluación basal del tiempo de congelación (# ratones que serán tratados). B) Evaluación del efecto agudo de una dosis de CBD (5 y 15 mg/kg) sobre el tiempo de congelación. C) Evaluación del efecto crónico de la administración de CBD (5 y 15 mg/kg) durante 7 días sobre el tiempo de congelación. * , valores de los ratones tratados con CBD significativamente diferentes en relación a aquellos de los ratones tratados con VEH (p<0,05). **, valores de los ratones tratados con CBD significativamente diferentes en relación a aquellos de los ratones tratados con VEH (p<0,001).

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5. Mallory J.E. Lofin Kimberly A. Babson Marcel O. Bonn-Miller. Cannabinoids as therapeutic for PTSD. Current Opinion in Psychology (2017). 14:78-83.

CONCLUSIONES

- El nuevo modelo crónico de TEPT propuesto produce una alteración notable y prolongada de la respuesta emocional (condicionamiento al miedo) de los animales expuestos.
- La administración aguda y crónica de CBD es capaz de reducir las alteraciones en la respuesta de condicionamiento al miedo de los ratones expuestos al modelo de TEPT.
- Son necesarios más estudios para mejorar la caracterización conductual y neurobiológica del modelo, así como para esclarecer los mecanismos que subyacen a los efectos farmacológicos del CBD.

Agradecimientos: Trabajo realizado en el marco de los proyectos financiados por el Plan Nacional Sobre Drogas (Ref. 2015/016) y las Redes Temáticas de Investigación Cooperativa en Salud (RETICS) (Red de Trastornos Adictivos (RTA), Ref. RD12/0028/0019) que obtienen sus fondos a través del Instituto de Salud Carlos III (Ministerio de Economía, Industria y Competitividad) y de FEDER (Fondo Europeo de Desarrollo Regional).

EVALUATION OF CANNABIDIOL EFFECTS IN A NEW ANIMAL MODEL OF POST-TRAUMATIC STRESS DISORDER

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SUMMARY AND OBJECTIVES

Post-traumatic stress disorder (PTSD) is an heterogeneous psychiatric disorder with difficult therapeutic management (1, 2) that makes necessary the development of new pharmacological strategies. For this reason, the design of new long-lasting PTSD-like animal models reflecting all the PTSD-related behavioural and neurochemical disturbances is indispensable to achieve it (3).

In the last years, several studies have demonstrated that cannabidiol (CBD), a constituent of *Cannabis Sativa* plant, can be useful for the treatment of anxiety disorders (4). Interestingly, its therapeutic usefulness for the management of PTSD-induced alterations has been recently proposed (5).

The main goals of this study are:

1. Validate and characterize a new PTSD-like animal model.
2. Evaluate the effects of CBD on the behavioural alterations (fear conditioning) of mice exposed to the PTSD-like animal model.

MATERIAL AND METHODS

Mice

Male C57BL/6J mice from Charles River (Lille, France) were housed in groups of 5 per cage (40 × 25 × 22 cm) under controlled conditions (temperature, 23 ± 2ºC; relative humidity, 60 ± 10 percent; 12-hour light/dark cycle, lights on from 8:00 am to 8:00 pm). The exposure to stressful stimuli to induce the PTSD-like model was initiated approximately 1 week after acclimatization to the animal room. Behavioural analyses were performed 1 hour after placing the home cage in the operant-task room to allow animals acclimatization. All the studies were conducted in compliance with the Spanish Royal Decree 53/2013, the Spanish Law 32/2007 and the European Union Directive of September 22, 2010 (2010/63/UE), regulating the care of experimental animals.

Drugs

Cannabidiol (CBD) obtained from STI Pharmaceuticals (Essex, UK) was dissolved in ethanol:cremophor:saline (1:1:18) immediately before the use to obtain the required doses (5 and 15 mg/kg). CBD was administered intraperitoneally (i.p.) 1 hour and 30 min before the behavioural evaluation.

PTSD-like animal model

Mice will be repeatedly exposed to different unpredictable stressful stimuli at different time points during 5 weeks.

- Electric shock: exposure to 1.0 mA scrambling shock during 10 seconds.
- Fox urine: exposure to fox urine during 15 minutes in a cage. A plastic tube containing a gauze impregnated in fox urine (Code Blue, Fox Urine Cover Scent, Ref. OA1105, 3 ml) is placed in the center of the cage.
- Movement restriction: acute movement restraint procedure for 15 minutes in a 50 ml falcon tube.
- Titled cage: during dark cycle, home cages will be tilted 30º for 12-14 hours.
- Wet bedding: during dark cycle, mice will be exposed to a cage with wet sawdust (material employed for bedding) for 12-14 hours.
- Food deprivation: during dark cycle, access to food will be restricted for 12-14 hours.

Fear conditioning

This test allows studying potential fear memory alterations by measuring the freezing response during a 5-min period of those mice exposed to the cage in which they previously received the electric shock. During the evaluation, no electric stimulus will be applied. The absence of apparent breathing and movement will be interpreted as freezing time. This paradigm will be carried out 4, 11 and 18 days after the last exposure to an electrical stimulus.

TIMELINE OF THE EXPERIMENTAL PROCEDURE TO INDUCE THE PTSD-LIKE ANIMAL MODEL IN C57BL/6J MICE

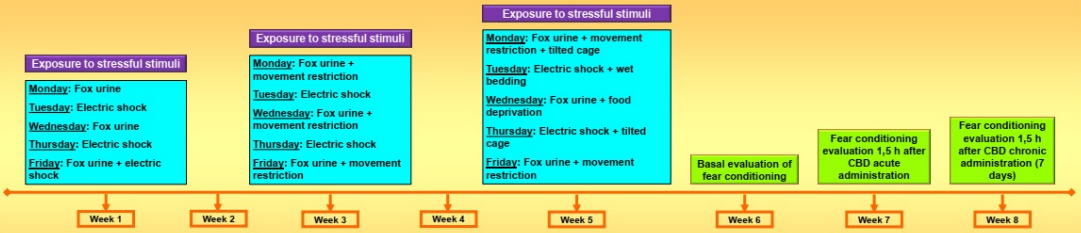

Figure 1. Time schedule of the PTSD-like animal model in C57BL/6J male mice. Stressful stimuli were applied in alternate weeks during a 5-week total period. Afterwards, emotional behaviour was evaluated through the fear conditioning paradigm under basal conditions (week 6) and when CBD was administered acutely (week 7) and chronically (week 8).

RESULTS - FEAR CONDITIONING EVALUATION

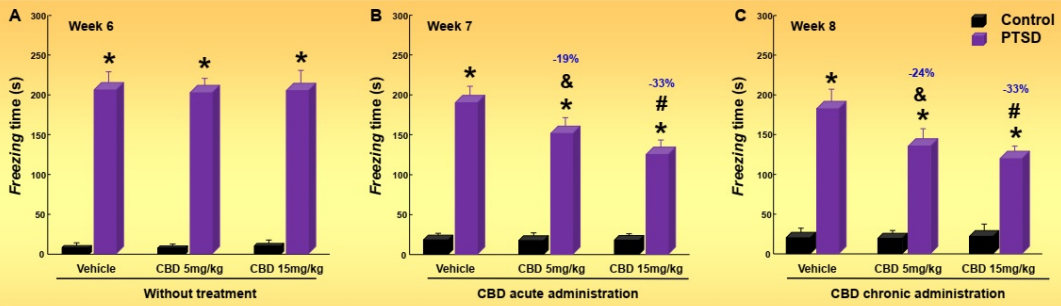

Figure 2. Fear conditioning evaluation on days 4, 11 and 18 after the last electric stimulus exposure. A) Basal evaluation of freezing time in the different experimental groups that will be treated with VEH or CBD. B) Evaluation of acute CBD treatment (5 and 15 mg/kg, i.p.) on freezing time. C) Evaluation of chronic CBD treatment (7 days, 5 and 15 mg/kg, i.p.) on freezing time. * values from animals exposed to the PTSD-like model that are significantly different from control mice ($p<0.001$). & values from CBD that are significantly different from VEH mice ($p<0.05$), # values from CBD that are significantly different from VEH mice ($p<0.001$).

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CONCLUSIONS

1. The new PTSD-like animal model induces noticeable and long-lasting emotional alterations.

2. Acute and chronic administration of CBD significantly reduces the freezing behaviour in those mice exposed to the PTSD-like model.

3. Further studies are guaranteed to improve the behavioural and neurobiological characterization of the PTSD-like animal model, and to elucidate the pharmacological mechanisms underlying CBD actions.

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