UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE PSICOLOGÍA

TESIS DOCTORAL

Factores Protectores y Biomarcadores de Deterioro Cognitivo en el Trastorno por Uso de Alcohol

Protective Factors and Biomarkers of Cognitive Impairment in Alcohol Use Disorder”

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Nerea Requena Ocaña

Directores

Fernando Rodríguez de Fonseca
Pedro Araos Gómez

Madrid

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"Vosotros nos traéis de nuevo a la luz de entre la oscuridad"

Paciente amnésico en tratamiento neuropsicológico

al cual tuve el placer de conocer un día

Dedicado, con cariño, a mis padres.

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RESUMEN

Factores protectores y biomarcadores de deterioro cognitivo en el Trastorno por Uso de Alcohol

La presente Tesis Doctoral tiene como objetivo describir cuáles son los factores que se asocian al deterioro cognitivo de los pacientes con Trastorno por Uso de Alcohol (TUA), que pueden actuar como elementos protectores y/o servir para su estratificación diagnóstica. La finalidad última de este trabajo consiste en demostrar que el consumo de cantidades elevadas de alcohol a lo largo de la vida acaba desencadenando un deterioro cognitivo que anticipa una demencia de inicio temprano, a pesar de que ciertos estudios científicos aseguran beneficios asociados a esta práctica. De este modo, se contribuye a dar un mensaje claro de salud pública para el desarrollo de políticas y estrategias de prevención óptimas para el deterioro cognitivo y la demencia relacionados con el TUA. Para lograr dicho objetivo, se han desarrollado cuatro estudios experimentales que se han centrado en la evaluación psicológica (entrevista PRISM) y neuropsicológica (FAB, MFE, MoCA, TAVEC, TMT B, FCRO y Dígitos), así como en la cuantificación de potenciales biomarcadores plasmáticos asociados al deterioro cognitivo (neurotrofinas, quimioquinas y neurofilamentos). Además, la presente Tesis Doctoral incluye una revisión sobre estudios clínicos y preclínicos sobre reserva cognitiva, entendida como un proceso activo para hacer frente un daño cerebral, en los trastornos por uso de sustancias y los potenciales biomarcadores asociados como trabajo teórico necesario para definir el marco de la investigación realizada.

En el primer trabajo se posiciona el nivel educativo (componente principal de la reserva cognitiva) como un factor mediador en la aparición del TUA y el desarrollo de deterioro cognitivo. En este estudio se demuestra que la neurotrofina 3 (NT-3) podría ser un potencial biomarcador de reserva cognitiva al encontrarse una disminución en los pacientes TUA con nivel educativo universitario, mientras que el Factor Neurotrófico Derivado del

Cerebro (BDNF) podría ser una señal de deterioro cognitivo ya que se encuentra disminuido en pacientes TUA con deterioro cognitivo. Además, estos demostraron ser potenciales biomarcadores de reserva cognitiva y de deterioro cognitivo, ya que eran capaces de distinguir entre pacientes con alta y baja reserva cognitiva, así como entre pacientes con y sin deterioro cognitivo.

El segundo estudio, tuvo como objetivo investigar el impacto de la reserva cognitiva sobre otras sustancias de abuso altamente comórbidas con el TUA. De este modo, se estimaron los efectos protectores derivados de un nivel educativo elevado sobre el trastorno por uso de cocaína, así como describir las diferencias de género en dicha población de estudio. Al igual que en el TUA, el nivel educativo es un factor mediador en la aparición y desarrollo del trastorno por uso de cocaína. En cuanto a las diferencias de género, las mujeres asisten en menor medida a los centros de tratamiento, consumen más medicación psicotrópica y tienen más trastornos de ansiedad y alimenticios que los hombres. En cambio, los hombres disponen más trastornos por uso de alcohol y cannabis.

En el tercer estudio se intentó avanzar en el conocimiento sobre la asociación entre neuroinflamación y deterioro cognitivo en los pacientes TUA. De este modo, encontramos como el Factor Trófico de Crecimiento Endotelial-Vascular (VEGFA) se encontraba aumentado en pacientes TUA con deterioro cognitivo severo. Además, VEGFA correlaciona de forma positiva y robusta con todas las quimioquinas evaluadas en pacientes TUA con deterioro cognitivo, lo cual no ocurre en los pacientes TUA sin deterioro cognitivo. Por lo tanto, teniendo en cuenta que los aumentos en VEGFA se han relacionado con el aumento de la permeabilidad de la barrera hematoencefálica y con la inclusión de mediadores inflamatorios en el sistema nervioso central, este parece ser un biomarcador fundamental a la hora de entender la neuroinflamación y el deterioro cognitivo asociado al consumo de alcohol.

Por último, en el cuarto estudio se pretendió demostrar que el TUA produce procesos neurodegenerativos tempranos que están asociados a la disfunción cognitiva observada mediante la cuantificación de un biomarcador nuevo de daño neuroaxonal –la cadena ligera de los neurofilamentos (NfL)-. De este modo, pudimos observar que las concentraciones de NfL en los pacientes con trastorno por uso de sustancias son muy similares a las encontradas en pacientes con demencia, sobre todo en los pacientes adictos con deterioro cognitivo severo. Es importante mencionar que los aumentos de NfL en plasma podrían deberse únicamente al TUA y no a los trastornos por uso de cocaína, cannabis o afectivos. Además, los NfL junto con el BDNF demostraron ser buenos predictores para discriminar entre pacientes con y sin deterioro cognitivo.

En conclusión, la presente Tesis Doctoral ha demostrado que el consumo problemático de alcohol a lo largo de la vida produce el detrimento de la función cognitiva. La fisiopatología subyacente al deterioro cognitivo observado podría consistir en la desregulación de los factores de crecimiento y la señalización inmune. Además, los pacientes TUA están desprovistos de la protección natural que ofrece un elevado nivel educativo y, por lo tanto, son aún más susceptibles de continuar abusando de sustancias y quedan más expuestos a las secuelas que producen las drogas a nivel cerebral. Como resultado, en los pacientes TUA se observan indicios de daño neuroaxonal que, con el paso del tiempo, podrían desencadenar en procesos neurodegenerativos similares a otros tipos de demencias.

SUMMARY

Protective factors and biomarkers of cognitive impairment in Alcohol Use Disorder

This Doctoral Thesis aims to describe the factors that are associated with cognitive deterioration in patients with Alcohol Use Disorder (AUD), which can act as protective elements and/or serve for diagnostic stratification. The ultimate purpose of this work is to demonstrate that the consumption of high amounts of alcohol throughout life leads to cognitive deterioration that anticipates early-onset dementia, even though certain scientific studies assure benefits associated with this practice. In this way, it contributes to giving a clear public health message for the development of optimal prevention policies and strategies for cognitive impairment and dementia related to alcohol use disorder. To achieve this objective, four experimental studies have been developed that have focused on psychological (PRISM interview) and neuropsychological evaluation (FAB, MFE, MoCA, VLTSC, TMT B, ROCF), as well as on the quantification of potential plasma biomarkers associated with cognitive impairment (neurotrophins, chemokines and neurofilaments). In addition, this Doctoral Thesis includes a review of clinical and preclinical studies of cognitive reserve, understood as an active process against brain damage, in substance use disorders and the possible associated biomarkers as theoretical work necessary to define the framework of the research carried out.

In the first study, the educational level (main component of cognitive reserve) is positioned as a mediating factor in the appearance of AUD and the development of cognitive impairment. This study demonstrates that neurotrophin 3 (NT-3) could be a potential biomarker of cognitive reserve, finding a decrease in AUD patients with university education, while Brain-Derived Neurotrophic Factor (BDNF) could be a signal of cognitive impairment since it is decreased in AUD patients with cognitive impairment. Furthermore, these proved to be possible biomarkers of cognitive reserve and cognitive impairment, since

they were able to distinguish between patients with high and low cognitive reserve, as well as between patients with and without cognitive impairment.

The second study aimed to investigate the impact of cognitive reserve on other substances of abuse highly comorbid with AUD. In this way, the protective effects derived from a high educational level on cocaine use disorder were estimated, as well as describing the gender differences in said study population. As in the AUD, educational level is a mediating factor in the appearance and development of cocaine use disorder. In terms of gender differences, women attend treatment centers to a lesser extent, consume more psychotropic medication and have more anxiety and eating disorders than men. In contrast, men have more alcohol and cannabis use disorders.

The third study attempted to advance knowledge about the association between neuroinflammation and cognitive impairment in AUD patients. In this way, we found how the Vascular Endothelial Growth Trophic Factor (VEGFA) was found in AUD patients with severe cognitive impairment. In addition, VEGFA positively and robustly correlates with all the chemokines evaluated in AUD patients with cognitive impairment, which does not occur in AUD patients without cognitive impairment. Therefore, considering that increases in VEGFA have been related to increased permeability of the blood-brain barrier and the inclusion of inflammatory mediators in the central nervous system, this seems to be a fundamental biomarker when it comes to understanding the Neuroinflammation and cognitive impairment associated with alcohol consumption.

Finally, the fourth study aimed to show that AUD produces early neurodegenerative processes that are associated with the cognitive dysfunction observed through the quantification of a new biomarker of neuroaxonal damage –neurofilament light chain (NfL)-. Thus, we were able to observe that NfL concentrations in patients with substance use disorders are very similar to those found in patients with dementia, especially in addicted patients with severe cognitive impairment. It is important to mention that increases

in plasma NfL might be due to AUD and not to cocaine, cannabis, or affective use disorders. Furthermore, NfL together with BDNF proved to be good predictors to discriminate between patients with and without cognitive impairment.

In conclusion, this Doctoral Thesis has shown that problematic alcohol consumption throughout life produces detrimental cognitive function. The pathophysiology underlying the observed cognitive impairment could consist of dysregulation of growth factors and immune signaling. In addition, AUD patients are devoid of the natural protection offered by a high level of education and, therefore, are even more likely to continue abusing substances and are more exposed to the sequelae produced by drugs at the brain level. As a result, signs of neuroaxonal damage are observed in AUD patients that, over time, could trigger neurodegenerative processes like other types of dementia.

Abreviaturas

BDNF: Brain-derived Neurotrophic Factor

DD: Dígitos directos (WAIS-IV)

DI: Dígitos Inversos (WAIS-IV)

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders Version IV

ELISA: Enzyme-Linked Immuno Sorbent Assay

ESTUDES: Encuesta sobre uso de drogas en Enseñanzas Secundarias en España

FAB: Frontal Assessment Battery

FCRO: Figura Compleja de Rey – Osterrieth

GABA: Ácido Gamma aminobutírico

HRP: Horse Radish Peroxidase

IMC: Índice de Masa Corporal

MCP-1: Monocyte Chemoattractant Protein 1

MFE: Memory Failure Everyday

MIP-1: Macrophage Inflammatory Protein 1

MoCA: Montreal Cognitive Assessment

NfLs: Neurofilamentos

NGF: Nerve Growth Factor

NMDA: N-metil-D-aspartato

NT-3: Neurotrofina 3

OEDT: Observatorio Europeo de las Drogas y las Toxicomanías

OMS: Organización Mundial de la Salud

PRISM: Psychiatric Research Interview for Substance and Mental Disorders

SDF-1: Stromal cell-Derived Factor 1

SIMOA: Single Molecule Protein Detection

SNC: Sistema nervioso central

TAVEC: Test de Aprendizaje Verbal España-Complutense

TMT: Trail Making Test

TRK: Tropomyosin related kinase

TUA: Trastorno por uso de Alcohol

TUS: Trastorno por uso de sustancias

VEGFA: Vascular Endothelial Growth Factor

1. Introducción

1.1. Trastornos por Uso de Alcohol y Demencia: dos grandes retos para la salud

1.1.1. Epidemiología del consumo de alcohol a nivel mundial

El alcohol etílico (etanol o simplemente alcohol) es una sustancia psicoactiva con propiedades altamente adictivas que ha sido utilizado ampliamente en muchas culturas durante milenios. Según la organización mundial de la salud (WHO, 2018), más de la mitad de la población mayor de 15 años consume alcohol en la Región de las Américas (54,1%) y la Región del Pacífico Occidental (53,8%). Sin embargo, los niveles más elevados de consumo de alcohol per cápita se observan en países de la Región de Europa (59,9%). Así los consumidores habituales beben alrededor de 32,8 gramos de alcohol puro al día, siendo las bebidas alcohólicas destiladas las más frecuentemente utilizadas (44,8%). No obstante, la prevalencia de intoxicaciones etílicas ha disminuido globalmente, aunque sigue siendo elevada particularmente en Europa del este. Además, en estas tres regiones mundiales se observa un inicio temprano del consumo de alcohol, produciéndose éste antes de los 15 años. Y aún peor, en Europa, un 43,8% de la población comprendida entre los 15 y los 19 años son bebedores habituales, afectando esta problemática a millones de adolescentes.

Además, el consumo nocivo de alcohol genera una carga sanitaria, social y económica considerable para el conjunto de la sociedad (World Health Organization, 2018). De este modo, se conoce que el consumo de alcohol es responsable de 3 millones de muertes anuales en el mundo, causando más de 200 enfermedades y trastornos (ej. enfermedades infecciosas, problemas de salud mental y comportamentales, traumatismos, cirrosis hepática, cáncer, enfermedades cardiovasculares, etc.). Los problemas derivados del consumo de alcohol constituyen el 5,1% de la carga mundial de morbilidad y lesiones (mayormente derivadas de accidentes de tráfico, actos de violencia y suicidios), provocando defunción y discapacidad a una edad temprana (20 a 39 años). Y no menos importante, su

consumo por la mujer gestante puede provocar graves alteraciones que van desde las malformaciones propias del síndrome alcohólico fetal a otras complicaciones menores que se denominan síndrome del espectro del alcohol fetal. Además, el consumo de alcohol no solo afecta a quien lo consume, sino que también puede perjudicar a otras personas (familiares, amigos, compañeros de trabajo y desconocidos).

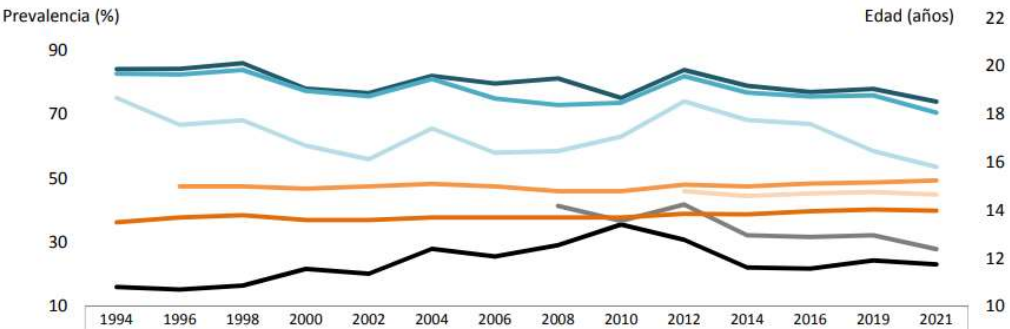
1.1.2. Epidemiología del consumo de alcohol en España

Según el Observatorio Español de las Drogas y las Adicciones (OEDA, 2019), entre la población de 15 a 64 años, el 93% ha consumido bebidas alcohólicas alguna vez en la vida, el 77% ha bebido alcohol en alguna ocasión durante los últimos 12 meses, el 63% ha consumido alcohol en el último mes y el 8,8% ha consumido alcohol a diario (cifra que ha aumentado 1,4 puntos con respecto al año 2017). De este modo, el consumo de alcohol supone un 35,2% de las admisiones a tratamiento (ha aumentado con respecto a 2018), aunque se encuentra presente en gran parte de los patrones de policonsumo. Además, es importante señalar que el 16,6% de la población ubicada entre los 55 y los 64 años afirma consumir alcohol diariamente, siendo muy superior a las tasas de consumo en otros rangos de edad (Figura 1). Este es un dato es de especial interés ya que a que el consumo elevado de alcohol se ha asociado con una aceleración del declive cognitivo en el envejecimiento y mayor riesgo de demencia (Woods et al., 2016).

	15-64 años			15-24 años			25-34 años			35-44 años			45-54 años			55-64 años		
	T	H	M	T	H	M	T	H	M	T	H	M	T	H	M	T	H	M
Alguna vez en la vida	93,0	95,5	90,4	88,7	90,2	87,1	93,9	96,1	91,7	93,1	95,3	90,9	94	96,1	91,9	93,8	98,3	89,3
Últimos 12 meses	77,2	82,7	71,6	79,3	81,9	76,7	79,2	86,1	72,3	77,9	82,7	73,0	76,7	81,6	71,9	73,6	81,6	65,6
Últimos 30 días	63,0	72,0	53,9	61,8	68,0	55,1	64,7	75	54,3	62,9	70,9	54,6	64,3	72,5	56,4	61	73,2	48,9
Diariamente en los últimos 30 días	8,8	14,2	3,4	1,3	1,9	0,6	4,0	6,1	1,8	8,4	13,5	3,1	10,9	18,0	4,0	16,6	26,8	6,5
Nunca	7,0	4,5	9,6	11,3	9,8	12,9	6,1	3,9	8,3	6,9	4,7	9,1	6,0	3,9	8,1	6,2	1,7	10,7

Figura 1. Prevalencia del consumo de alcohol en la población de 15-64 años. Extraído del Observatorio Español de las Drogas y las Adicciones (OEDA, 2019).

Por otro lado, la prevalencia del consumo de alcohol entre los jóvenes continúa siendo extremadamente elevada (Figura 2), cuyo inicio y uso habitual se produce de manera muy precoz (14 y 15 años, respectivamente). Según la Encuesta sobre uso de drogas en Enseñanzas Secundarias en España (ESTUDES, 2021), entre los jóvenes de 14 a 18 años el 73,9% ha consumido alcohol alguna vez en la vida, el 70,5% ha consumido alcohol en el último año y el 53,6% ha consumido alcohol en el último mes. Además, un 27,9% de los jóvenes entre 14 a 18 años ha hecho consumo en atracón (*binge drinking*, consumo de 5 o más vasos de bebidas alcohólicas en un intervalo aproximado de dos horas) en los últimos 30 días. Este problema es especialmente relevante teniendo en cuenta la vulnerabilidad del sistema nervioso central (SNC) en desarrollo ante posibles agentes externos como puede ser el consumo de alcohol en la adolescencia (Tapia-Rojas et al., 2017, 2018). De este modo, se ha descrito que el inicio temprano del consumo de alcohol parece ser un factor de riesgo para un funcionamiento neuropsicológico posterior más pobre en los adultos jóvenes (Nguyen-Louie et al., 2017). Precisamente, la vinculación del alcohol con el deterioro cognitivo que puede observarse en adultos que comenzaron con consumo intensivo cuando eran adolescentes o adultos jóvenes, es un problema de primera magnitud que no ha sido abordado correctamente, y es el núcleo central de esta investigación. De hecho, como vamos a ver en el siguiente capítulo, la demencia es otro de los retos de salud pública más importantes de las sociedades modernas, y el alcohol ha sido postulado recientemente como un contribuyente neto a todos los tipos de demencias clínicamente relevantes (Sabia et al. 2018)



	1994	1996	1998	2000	2002	2004	2006	2008	2010	2012	2014	2016	2019	2021
Alguna vez en la vida	84,1	84,2	86,0	78,0	76,6	82,0	79,6	81,2	75,1	83,9	78,9	76,9	77,9	73,9
Últimos 12 meses	82,7	82,4	83,8	77,3	75,6	81,0	74,9	72,9	73,6	81,9	76,8	75,6	75,9	70,5
Últimos 30 días	75,1	66,7	68,1	60,2	56,0	65,6	58,0	58,5	63,0	74,0	68,2	67,0	58,5	53,6
Borracheras últimos 30 días	16,1	15,3	16,5	21,7	20,2	28,0	25,6	29,1	35,6	30,8	22,2	21,8	24,3	23,2
Binge drinking últimos 30 días								41,4	36,7	41,8	32,2	31,7	32,3	27,9
Edad media de inicio consumo	13,5	13,7	13,8	13,6	13,6	13,7	13,7	13,7	13,7	13,9	13,8	14,0	14,0	14,0
Edad media de consumo alcohol semanal		15,0	15,0	14,9	15,0	15,1	15,0	14,8	14,8	15,1	15,0	15,1	15,2	15,2
Edad primera borrachera										14,8	14,6	14,7	14,8	14,7

Figura 2. Prevalencia y edades de inicio del consumo de alcohol entre los jóvenes de 14-18 años. Extraído de la Encuesta sobre uso de drogas en Enseñanzas Secundarias en España (ESTUDES).

1.1.3. Epidemiología de la demencia a nivel mundial

La demencia es un síndrome de naturaleza crónica o progresiva que conduce a un deterioro de la función cognitiva y no es considerado una consecuencia inevitable del envejecimiento. La demencia tiene importantes impactos psicológicos, sociales y económicos a nivel mundial, no solo para la persona que lo padece, sino también para sus cuidadores, familiares y sociedad en general (World Health Organization, 2017). La demencia constituye la séptima causa principal de muerte entre todas las enfermedades y es una de las principales causas de discapacidad y dependencia entre las personas mayores a nivel mundial. La etiología de esta enfermedad es el resultado de una variedad de enfermedades y lesiones que afectan al cerebro, siendo la enfermedad de Alzheimer la más común (60-70% de los casos). Actualmente, más de 55 millones de personas viven con demencia en todo el mundo y hay casi 10 millones de casos nuevos cada año. No obstante, esta dolencia no afecta exclusivamente a personas mayores, sino que la demencia de inicio joven (definida como el inicio de los síntomas antes de los 65 años) representa hasta el 9% del total de los casos (Figura 3).

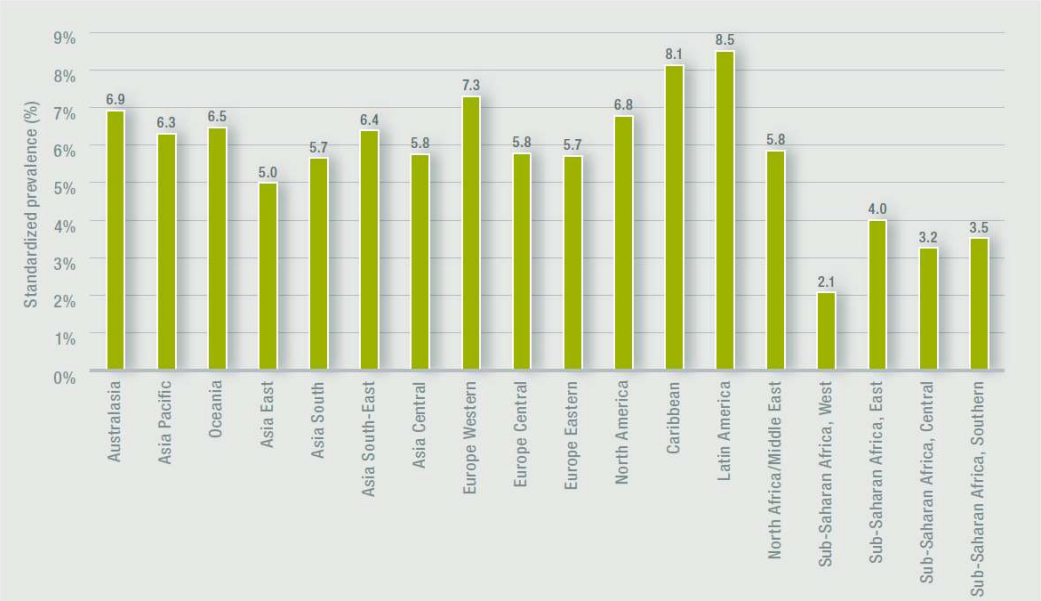


Figura 3. Prevalencia de demencia en personas de 60 años o más en distintas regiones del mundo según la OMS (World Health Organization, 2017).

Por todos estos motivos la demencia se ha considerado como una prioridad de salud pública, posicionándose como una de las principales enfermedades del siglo XXI. En los próximos 30 años se vaticina un notable aumento de la prevalencia de demencia, pudiendo llegar a alcanzar la cifra de 129 millones de personas afectadas para el año 2050. Por eso la Asamblea Mundial de la Salud ha respaldado un Plan de acción mundial sobre la respuesta de salud pública a la demencia 2017-2025 para el desarrollo de una respuesta global coordinada para abordar de manera efectiva la problemática de esta enfermedad (World Health Organization, 2017). No obstante, una reciente noticia de la OMS informa que el mundo no está abordando el reto de la demencia: solo una cuarta parte de los países cuentan con una política, estrategia o plan nacional de apoyo a las personas que sufren esta dolencia.

Como hemos indicado antes, los estudios prospectivos y retrospectivos en cohortes europeas claramente identifican al consumo de alcohol como un contribuyente neto en el desarrollo de demencia (Sabia et al., 2018; Schwarzinger et al., 2018 a,b). Teniendo en cuenta que la población goza de una longevidad cada vez mayor, y que el consumo en edades jóvenes en patrón intensivo se ha extendido, siendo alarmantemente elevados, es

necesario investigar la relación entre alcohol y demencia por su enorme carga social, y la posibilidad de realizar intervenciones preventivas.

1.1.4. Epidemiología de la demencia en España

De acuerdo con el Plan Integral de Alzheimer y otras Demencias (Ministerio de Sanidad, 2019) la prevalencia de esta enfermedad ronda el 0,05% entre las personas de 40 a 65 años; 1,07% entre los 65-69 años; 3,4% en los 70-74 años; 6,9% en los 75-79 años; 12,1% en los 80-84; 20,1 en los 85-89; y 39,2% entre los mayores de 90 años. El número de personas afectadas en España supera las 700.000 personas de más de 40 años y se estima que el número de enfermos se duplicará en 2050. Ante el impacto social y económico que plantea esta problemática a la sociedad española, se ha creado una planificación que contribuye a impulsar avances políticos, sociales, sanitarios y científicos en los próximos años.

1.2. La adicción al alcohol y sus comorbilidades

Según la *American Society of Addiction Medicine*, la adicción es una enfermedad crónica relacionada con la inhabilidad para abstenerse, el deterioro del control de impulsos, el ansia de consumo (*craving*), la disminución del reconocimiento de los problemas comportamentales y en las relaciones interpersonales, así como una respuesta emocional disfuncional (American Society of Addiction Medicine, 2011). Además, la adicción implica ciclos de recaída y remisión que, sin tratamiento, puede resultar en discapacidad o muerte prematura. Dicha enfermedad se rige por mecanismos neurobiológicos que no sólo tienen que ver con las áreas relacionadas con la recompensa, como el núcleo accumbens, el córtex cingulado anterior, el prosencéfalo y la amígdala, sino que también se relaciona con áreas prefrontales del cerebro relacionadas con la alteración del control de impulsos, el juicio y la búsqueda disfuncional de la recompensa (American Society of Addiction Medicine, 2011).

1.2.1. Neurobiología de la adicción

La comprensión de la neurobiología de la adicción ha progresado a través de la neuropsicofarmacología mediante el estudio de modelos animales y, más recientemente, a través de la neuroimagen mediante el estudio de imágenes funcionales cerebrales en personas con adicción. De este modo, los modelos animales del ciclo de la adicción han podido ser respaldados en humanos a través de cartografía funcional cerebral (imágenes de resonancia magnética funcional o de tomografía de emisión de positrones) en pacientes con uso problemático de sustancias.

Por norma general, todas las drogas de abuso modifican la actividad de liberación de dopamina de las neuronas del área tegmental ventral, actuando directamente sobre ellas o indirectamente sobre las neuronas que regulan las células dopaminérgicas, capaces de activarlas o de inhibirlas. La respuesta celular aguda tras el consumo de una droga supone la modificación de una cadena de señalización intracelular que afecta finalmente a la excitabilidad de la neurona (ej., despolarizándola o inhibiéndola), y a la expresión de genes que condicionan su fenotipo funcional (Di Chiara G, 1988). Sin embargo, si la estimulación se mantiene en el tiempo de forma crónica, pueden conducir a dos tipos de fenómenos: a) la tolerancia, en la cual se ponen en marcha mecanismos homeostáticos de contrarrespuesta que conducen a la atenuación del estímulo producido por la droga (ej., una disminución del efecto sedante de un opiáceo), b) la sensibilización, en la cual se produce una magnificación de la respuesta evocada por la droga, que crece con cada exposición (ej., la sensibilización de la actividad locomotora observada tras la exposición repetida a cocaína). Es importante mencionar que cuando se produce la retirada abrupta de la droga consumida crónicamente, estos mecanismos de contrarrespuesta que conducen a tolerancia se manifiestan como una abrupta respuesta opuesta a la generada por la exposición aguda a la droga: la respuesta de abstinencia (Nestler 2001 & 2005). Por ejemplo, mientras que la exposición aguda al alcohol provoca una potenciación de GABA y una disminución de liberación de glutámico

en la exposición aguda que resulta en un efecto de sedación; si se retira abruptamente el alcohol tras el desarrollo de tolerancia por consumo crónico, aparecerá un síndrome de abstinencia dramático, con irritabilidad, convulsiones y delirio alucinatorio producido por la hiperexcitabilidad (contrarrespuesta) ante la depresión de la señalización de GABA y la hiperactividad glutamatérgica asociada.

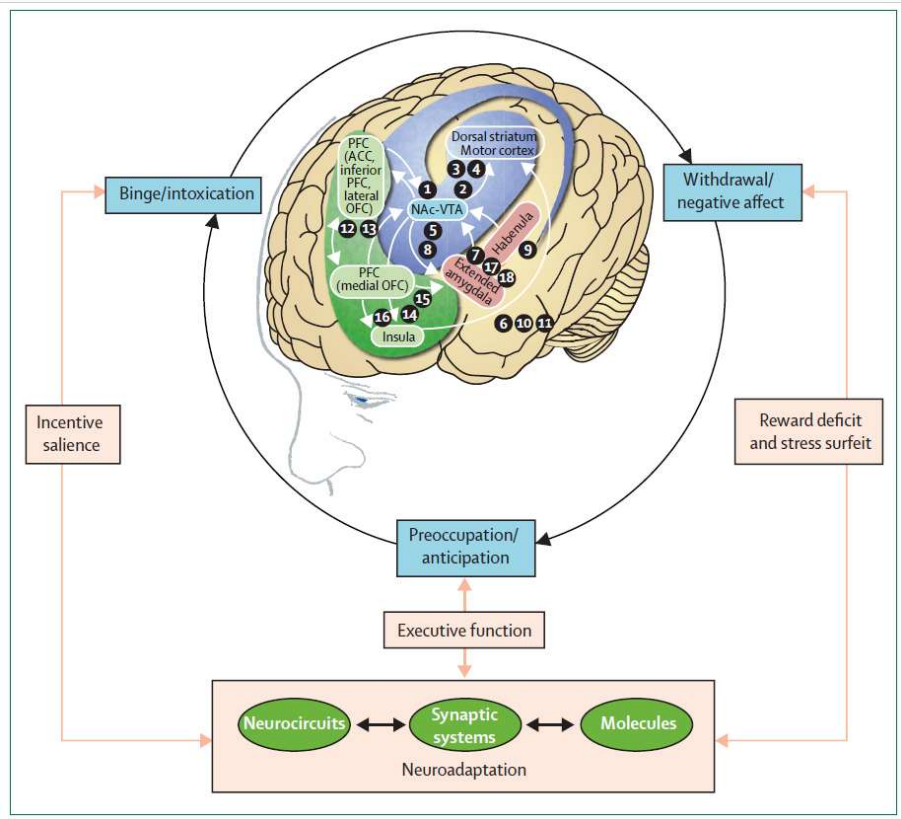


Figura 4. Circuitos neuronales de las etapas que caracterizan la adicción a las drogas. Los colores corresponden en azul a la etapa de atracón/intoxicación (recompensa y prominencia del incentivo: ganglios basales), en rojo a la etapa de retraimiento/afecto negativo (estados emocionales negativos y estrés: amígdala extendida y habénula) y en verde a la etapa de preocupación/anticipación (ansia, impulsividad y función ejecutiva: PFC, ínsula y alocorteza). Figura extraída de Koob & Volkow (2016).

El trastorno adictivo a drogas se caracteriza por tener un curso temporal en el que se suceden una serie de etapas definidas por fenómenos característicos que afectan a la relación existente entre el comienzo del uso recreativo de una sustancia y el final de pérdida de control y abuso de la misma, y que reclutan secuencialmente distintos tipos de circuitos

cerebrales (Figura 4) (Koob & Volkow, 2016; Uhl et al., 2019): a) la etapa impulsiva de atracón / intoxicación impulsada por los ganglios basales (circuitos de recompensa), b) la etapa de abstinencia / afecto negativo impulsada por la amígdala extendida y sus conexiones con los ejes neuroendocrinos y el sistema nervioso autónomo, c) la etapa de preocupación / anticipación impulsada por la corteza prefrontal y sus conexiones subcorticales.

Los mecanismos neurobiológicos de la etapa de atracón / intoxicación implican la combinación déficits en el sistema de recompensa y una prominencia de incentivos exagerada. La primera etapa en el camino de la adicción es la aparición de la activación del sistema de recompensa al consumirse una droga de abuso. El sistema de recompensa es un complejo circuito formado por proyecciones ascendentes desde núcleos mesencefálicos a regiones ventrales de los ganglios basales y la corteza prefrontal, y las correspondientes proyecciones descendentes. Dentro del sistema de recompensa, el circuito principal al que se atribuyen los efectos gratificantes de las drogas de abuso ha sido el configurado por el sistema de dopamina ascendente que conecta el área tegmental ventral con el núcleo accumbens. De hecho, la liberación de dopamina y péptidos opioides durante ingesta aguda de alcohol y otras drogas está asociada a la sensación de euforia placentera ("*high*"). Sin embargo, atribuir solamente a la dopamina el poder adictivo de una droga es una concepción limitada, ya que en el circuito de recompensa intervienen una gran variedad de neuromoduladores, como el GABA, el glutámico, la serotonina y la acetilcolina (Figura 5). Además, estímulos previamente neutrales que se asocian con la disponibilidad de la droga pueden activar el sistema de recompensa, convirtiéndose en reforzadores secundarios y promoviendo la formación de hábitos que fomentan la búsqueda compulsiva y las conductas de autoadministración de drogas. Esto se produce porque desde la amígdala y el núcleo accumbens se generan unas rutinas de comportamiento (hábitos compulsivos) que se implementan en los circuitos subcorticales de los ganglios basales (estriato-palidales) y que garantizan la búsqueda, consecución y consumo de drogas. Estos comportamientos

altamente estructurados escapan con el tiempo al control ejecutivo consciente ejercido desde la corteza prefrontal y se convierten en automatismos que se ponen en marcha ante la aparición de señales contextuales predictivas de la presencia de la droga, su contexto, o de la inminente entrada en abstinencia (Koob & Volkow, 2016; Lüscher C, Robbins TW, 2020; Uhl et al., 2019).

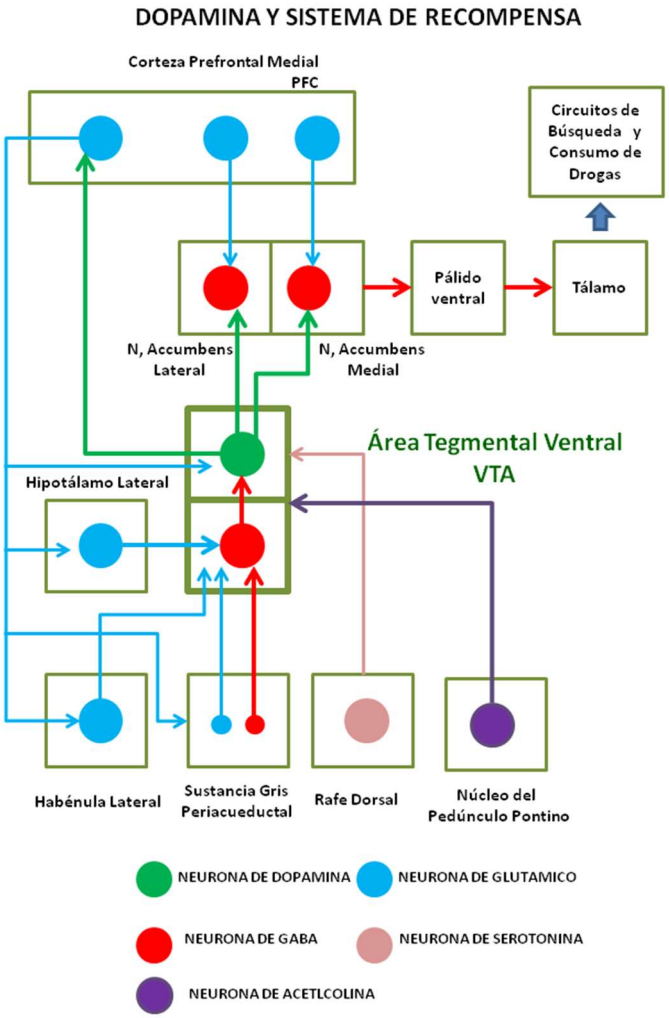


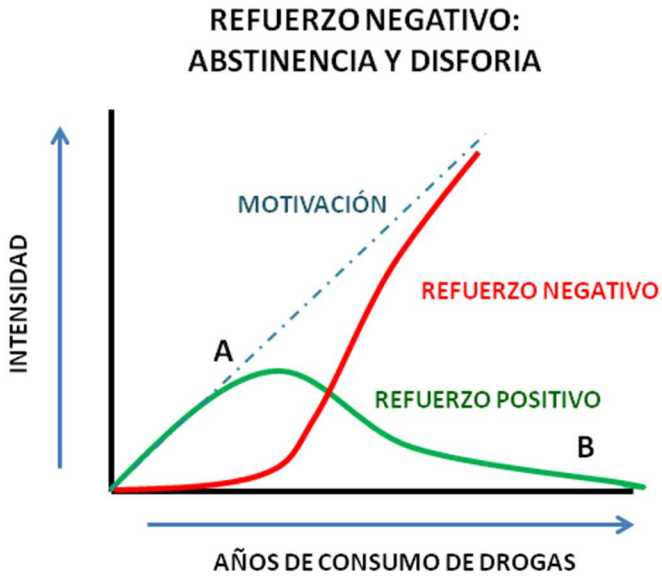
Figura 5. Esquema simplificado del circuito de recompensa mesotelencefálico. Desde el área del tegmento ventral (VTA), las neuronas dopaminérgicas (en verde), proyectan al núcleo accumbens y a la corteza prefrontal, portando la señal de refuerzo positivo activada por las drogas de abuso (detección y anticipación de la droga). Desde el núcleo accumbens, vía pálido y tálamo, se orquesta la organización del aprendizaje y ejecución del comportamiento de búsqueda de droga. Estas neuronas de la VTA son inhibidas por interneuronas GABA en la propia VTA. Una serie de proyecciones, tanto a las neuronas

dopaminérgicas como a las interneuronas GABA, orquestan la activación de las neuronas de dopamina. Por ejemplo, la nicotina puede activar directamente las neuronas de dopamina a través de receptores nicotínicos, mientras que los opiáceos o el al alcohol lo hacen inhibiendo las interneuronas de GABA directamente (opiáceos) o indirectamente (vía circuito corteza prefrontal-sustancia gris periacueductal). El hipotálamo lateral activa directamente las interneuronas GABA provocando aversión al fomentar la inhibición de estas interneuronas de GABA. El circuito corteza prefrontal-habénula lateral-VTA es esencial para la interpretación aversiva o reforzadora de las experiencias estresantes o novedosas.

La etapa de abstinencia / afecto negativo se define como irritabilidad crónica, dolor emocional, malestar, disforia, alexitimia, estados de estrés y pérdida de motivación por recompensas naturales. A medida que el consumo de drogas se repite y se intensifica, se produce una atenuación de la respuesta dopaminérgica, y empiezan a emerger sentimientos afectivos negativos, es decir, se desencadena una contrarrespuesta a la tolerancia (Figura 6). De este modo, se produce la desregulación del sistema cerebral de gestión del estrés compuesto por el circuito funcional formado por el complejo amigdalar (en especial su núcleo eferente, el núcleo central) y el llamado eje hipotalámico-pituitario-suprarrenal. Así, la respuesta comportamental, hormonal o del sistema nervioso autónomo se orquesta a través de las proyecciones amigdalofugales desde el núcleo central de la amígdala utilizando principalmente el factor liberador de corticotropina (CRF). Por lo tanto, tras el consumo de drogas a lo largo del tiempo, estos circuitos se hipersensibilizan produciendo ansiedad, afecto negativo y necesidad de consumo, fomentándose el refuerzo negativo: evitar dejar de consumir para no exponerse a las consecuencias afectivas negativas que supone la hiperactividad de la amígdala durante la abstinencia (Koob & Volkow, 2016; Koob GF, 2008; Uhl et al., 2019).

La etapa de preocupación / anticipación es la etapa en la que el individuo restablece la conducta de búsqueda de drogas, por lo que es clave para la recaída y define la adicción como un proceso crónico y recidivante. Los déficits en la función ejecutiva derivados del consumo de drogas perpetúan la falta de control de la prominencia de incentivos cuando se

presenta una señal sobresaliente a través de la desregulación de las redes neuronales glutamatérgicas, GABAérgicas y dopaminérgicas en la corteza prefrontal. Además, la disminución de la actividad de la corteza frontal interfiere en la toma de decisiones, la autorregulación, el control inhibitorio y la memoria de trabajo a través de una actividad GABAérgica interrumpida, produciendo la incapacidad de inhibir el comportamiento desadaptativo derivado del consumo de sustancias (Koob & Volkow, 2016; Uhl et al., 2019).



AREA	A	B
N. ACCUMBENS	↑ DOPAMINA, PÉPTIDOS OPIOIDES	↓ DOPAMINA, PÉPTIDOS OPIOIDES
VTA	↑ TH, BDNF, PKA	↓ TH, BDNF, PKA
CORTEX PREFRONTAL	↑ DOPAMINA, GABA	↑ GLUTAMATO, NORADRENALINA ↓ DOPAMINA
AMIGDALA	↑ GABA, NPY	↑ CRF, DINORFINA NORADRENALINA ↓ GABA NPY

Figura 6. Diagrama que muestra la historia natural de la adicción desde la perspectiva de la naturaleza motivacional del consumo de drogas (Hipótesis de la desregulación hedónica). En la primera fase (A) las acciones de las drogas producen un refuerzo positivo y se establecen asociaciones contextuales (reforzador secundario) dado que activan el circuito de recompensa, liberando dopamina y

péptidos opioides. Con el paso del tiempo (B) se produce una contrarrespuesta que conduce a una desactivación del sistema de recompensa, bajando la liberación de dopamina, mientras se activan los circuitos amigdalofugales de la ansiedad y la disforia, por lo que predomina el refuerzo negativo, al potenciarse el CRF y la dinorfina, así como la liberación de noradrenalina. A por tanto es un periodo de búsqueda de droga motivado positivamente (búsqueda del placer o del afecto positivo), mientras que B es un periodo de búsqueda de droga para aliviar un proceso endógeno disfórico y negativo (refuerzo negativo). Abreviaturas: TH, tirosina hidroxilasa; BDNF, factor neurotrófico derivado del cerebro; PKA, proteín kinasa A; CRF, factor liberador de corticotrofina; NPY, neuropéptido Y.

1.2.2. Trastornos relacionados con el uso de alcohol

Según el Instituto Nacional sobre el Abuso de Alcohol y Alcoholismo (NIAAA, 2021), el consumo excesivo de alcohol corresponde al consumo de 4 bebidas estándar o más para las mujeres, y 5 bebidas estándar o más para los hombres en aproximadamente 2 horas. Este patrón de ingesta de alcohol llamado consumo en atracón o *binge drinking* se caracteriza por elevar rápidamente el nivel de alcohol en la sangre por encima de los niveles de 0,08%, o 0,08 gramos de alcohol por decilitro.

Los efectos producidos por la intoxicación por ingesta de alcohol se caracterizan por cambios psicológicos y comportamentales clínicamente significativos, pudiendo ocasionar hasta el coma etílico y el riesgo vital en la persona (American Psychiatric Association, 2013). La evidencia del consumo de alcohol se puede obtener a partir del olor del alcohol en el aliento de la persona, al recabar información del individuo o un observador o realizando análisis toxicológicos en sangre, orina o aliento. Según el Manual diagnóstico y estadístico de los trastornos mentales en su edición 5 (DSM-5) elaborado por la Asociación Estadounidense de Psiquiatría, los síntomas clínicos manifestados por la intoxicación al alcohol se presentan de forma detallada en la Tabla 1:

Tabla 1. Criterios diagnósticos para intoxicación por alcohol según el DSM-5.

A. Consumo de alcohol reciente

B. Comportamiento y cambios psicológicos inadecuados y problemáticos notorios que aparecen durante o después del consumo de alcohol (ej. agresividad, inestabilidad emocional, deterioro del juicio, comportamiento sexual desinhibido, etc.)

C. Durante o poco después del consumo de alcohol se da uno o más síntomas siguientes:
   a) Habla disártrica.
   b) Descoordinación.
   c) Marcha insegura.
   d) Nistagmo.
   e) Alteración de la atención y la memoria.
   f) Estupor o coma.

D. Los signos o síntomas no se explican mejor por otro trastorno mental o afección médica.

El consumo excesivo de alcohol puede conducir a un Trastorno por Uso de Alcohol (TUA) que se define como un patrón problemático de consumo de alcohol acompañado de un deterioro o angustia clínicamente significativos. El TUA requiere que se cumplan ≥2 criterios diagnósticos en un período de 12 meses. Leve equivale a 2-3 criterios; moderado, 4-6 criterios; y severos, 7-11 criterios (American Psychiatric Association, 2013). Los criterios diagnósticos para el TUA se presentan de forma detallada en la Tabla 2:

Tabla 2. Criterios diagnósticos para TUA según el DSM-5.

A. Consumo problemático de alcohol que provoca deterioro o malestar clínicamente significativo y que se manifiesta al menos por dos de los siguientes:
   1. A menudo, el alcohol se ingiere en cantidades mayores o durante un período más prolongado de lo previsto.
   2. Existe un deseo persistente o esfuerzos infructuosos por reducir o controlar el consumo de alcohol.
   3. Se dedica mucho tiempo a las actividades necesarias para obtener alcohol, consumir alcohol o recuperarse de sus efectos.
   4. Deseo o un fuerte deseo o urgencia de consumir alcohol.

5. El consumo recurrente de alcohol resulta en el incumplimiento de las principales obligaciones del papel en el trabajo, la escuela o el hogar.
6. Consumo continuo de alcohol a pesar de tener problemas sociales o interpersonales persistentes o recurrentes causados o agravados por los efectos del alcohol.
7. Las actividades sociales, ocupacionales o recreativas importantes se abandonan o reducen debido al consumo de alcohol.
8. Consumo recurrente de alcohol en situaciones en las que es físicamente peligroso.
9. El consumo de alcohol se continúa a pesar de saber si tiene un problema físico o psicológico persistente o recurrente que probablemente haya sido causado o agravado por el alcohol.
10. Tolerancia, definida por cualquiera de los siguientes:
    a) Necesidad de cantidades notablemente mayores de alcohol para lograr la intoxicación o los efectos deseados.
    b) Un efecto notablemente disminuido con el uso continuo de la misma cantidad de alcohol.
11. Abstinencia, manifestada por cualquiera de los siguientes:
    a) El síndrome de abstinencia característico del alcohol (consulte los criterios A y B de los criterios establecidos para la abstinencia de alcohol).
    b) Alcohol (o una sustancia estrechamente relacionada, como benzodiazepina) que se toma para aliviar o evitar los síntomas de abstinencia.

El inicio de la ingesta de alcohol está íntimamente asociado con factores ambientales como la aceptación cultural hacia la bebida, las experiencias personales relacionadas con el consumo alcohol y el afrontamiento del estrés. Sin embargo, la vulnerabilidad al abuso de alcohol está determinada por un complejo abanico de factores genéticos, que representan un 50-60% del riesgo. De este modo, polimorfismos de un solo nucleótido que afectan a enzimas relacionadas con el metabolismo del alcohol como la alcohol dehidrogenasa (ADH) y la aldehído dehidrogenasa (ALDH), especialmente los genes ADH4 y ALDH1A1, se han asociado con la susceptibilidad al alcoholismo (J. Y. Yang et al., 2014).

Después de unas horas o pocos días del cese o disminución de la ingesta excesiva y continuada de alcohol, y su concentración en sangre disminuye significativamente, se puede producir el síndrome de abstinencia (American Psychiatric Association, 2013). Los síntomas alcanzan su máxima intensidad durante el segundo día de la abstinencia, sin embargo, síntomas como ansiedad, insomnio y la disfunción autonómica pueden presentarse de 3 a 6 meses después, aunque con menor intensidad. Además, puede aparecer un estado confusional, cambios en la conciencia y la cognición, así como alucinaciones visuales, táctiles o auditivas; es decir, el desarrollo de un delirium tremens durante la abstinencia del alcohol. Los criterios diagnósticos para la abstinencia al alcohol se detallan en la Tabla 3:

Tabla 3. Criterios diagnósticos para la abstinencia de alcohol según el DSM-5.

A. Interrupción o disminución de la ingesta de alcohol que ha sido muy intenso y prolongado.
B. Después de pocas horas o pocos días de la interrupción o disminución del consumo de alcohol aparecen dos o más de los siguientes: a) Hiperactividad del sistema nervioso autónomo (ej. sudoración o taquicardia). b) Temblor de manos. c) Insomnio. d) Nauseas o vómitos. e) Alucinaciones visuales, auditivas o táctiles transitorias. f) Agitación psicomotora. g) Ansiedad. h) Convulsiones tónico-clónicas
C. Los síntomas anteriores provocan un malestar clínicamente significativos o deterioro en la funcionalidad en lo social, laboral u otras áreas
D. Los signos o síntomas no se explican mejor por otro trastorno mental o afección médica.

1.2.3. Comorbilidades con otros trastornos por uso de sustancias.

Asimismo, los pacientes abusadores de alcohol suelen presentar adicionalmente otro TUS comórbido (en torno al 45%), siendo el trastorno por uso de cocaína el más frecuente seguido del trastorno por uso de cannabis (García-Marchena et al., 2017; García-Marchena, Pizarro, et al., 2020). Del mismo modo, en los pacientes que abusan de la cocaína los TUS más frecuentes son por uso de alcohol y cannabis (McHugh et al., 2018; Sanvicente-Vieira et al., 2019). Es importante señalar que el trastorno por uso de cocaína se ha establecido como la droga estimulante ilegal más consumida en Europa, ocupando España el segundo lugar en la Unión Europea (EMCDDA, 2019). De este modo, en España, el 10,9% de los adultos (15-64 años) ha consumido cocaína a lo largo de su vida, el 2,5% en el último año y 1,1% en el último mes (OEDA, 2019). Este es un dato de especial relevancia teniendo en cuenta de que los pacientes con consumo crónico de cocaína experimentan una elevada comorbilidad psiquiátrica, especialmente por trastornos del estado de ánimo y de personalidad (Pedraz et al., 2015), así como indicios de deterioro cognitivo. De este modo, algunos estudios sugieren que el consumo prolongado de cocaína produce el deterioro en la atención, el aprendizaje, la memoria verbal (Potvin et al., 2014) y las funciones ejecutivas (García-Marchena et al., 2018; Potvin et al., 2014; Schulte et al., 2014). Además, los pacientes abstinentes con trastorno por uso de cocaína pueden experimentar una recuperación más rápida que los pacientes consumidores de alcohol, alcanzando alrededor de los 5 meses la mejora parcial de los procesos cognitivos (Potvin et al., 2014). En contraposición, un estudio meta-analítico sugiere que la evidencia actual no respalda la opinión de que el consumo crónico de cocaína este asociado con amplios déficits cognitivos, a pesar de que se encuentren diferencias a nivel cerebral y metabólico en estos pacientes (Frazer et al., 2018). Una posible explicación es que quizás esas alteraciones cognitivas se produzcan únicamente por el consumo de alcohol que presentan de forma concomitante los pacientes abusadores de cocaína (Blanco-Presas et al., 2018).

1.2.4. Comorbilidades con otros trastornos psiquiátricos

El Trastorno por Uso de Alcohol presenta una elevada prevalencia de comorbilidad psiquiátrica (en torno al 65%) que se puede producir durante, antes o después de un periodo de consumo excesivo o prolongado del alcohol (Garcia-Marchena, Araos, et al., 2017; García-Marchena, Maza-Quiroga, et al., 2020). Entre los trastornos mentales que con frecuencia se diagnostican concomitantemente con el TUA son: el trastorno depresivo mayor, la distimia, el trastorno bipolar, la ciclotimia, el trastorno de ansiedad generalizada, los ataques de pánico, trastornos psicóticos, trastorno de déficit de atención e hiperactividad, trastorno de la conducta en la infancia y trastorno de la personalidad antisocial y límite (Chen et al., 2011; Fein, 2015; Preuss et al., 2018). Se dice que un trastorno es inducido por el alcohol cuando se cumplen todos los criterios diagnósticos para ese trastorno; el episodio ocurre durante un periodo de ingesta de alcohol excesivo o en las cuatro semanas posteriores al cese del consumo; y cuando los síntomas son claramente excesivos respecto a los efectos esperados de la intoxicación y/o la abstinencia. En cambio, se considera como trastorno primario cuando el episodio se produce cuando el sujeto no consume alcohol (abstinencia) o cuando la cantidad del mismo no es suficiente para producir intoxicación o abstinencia (consumo ocasional), el episodio comienza dos semanas antes del inicio del consumo de alcohol o cuando el episodio comienza durante el consumo excesivo de alcohol y continúa durante al menos cuatro semanas o más después del cese del consumo (Koten et al., 2001). La comorbilidad psiquiátrica en los trastornos por uso de sustancias es importante ya que estos pacientes pueden adicionalmente presentar el deterioro cognitivo del propio trastorno psiquiátrico comórbido lo que puede ser un agravante serio para el pronóstico de estos pacientes (Fernandes et al., 2014). De este modo, aunque algunos estudios sugieren que el consumo excesivo y prolongado de alcohol producen por si solos el deterioro en la cognición, la comorbilidad psiquiátrica afectiva

podría incrementar la discapacidad funcional después de haber cesado su consumo (Lee et al., 2015).

1.3. Deterioro cognitivo leve, demencia y alcohol

El consumo de un tóxico como el alcohol puede resultar en un daño tisular que puede afectar a múltiples órganos, incluyendo el cerebro. En este último, las lesiones pueden ser irreversibles generando déficits funcionales severos, que se presentan como una pérdida de las capacidades cognitivas, que puede llegar a ser tan grave como una demencia. Es importante definir por tanto qué tipos de daño cerebral puede generar el alcohol y ubicarlo en el contexto de las enfermedades cerebrales que pueden generar déficits en la cognición.

1.3.1. Definición y criterios diagnósticos

Se denomina daño cerebral adquirido a las lesiones del SNC que se producen a partir de la infancia y que no son derivadas del desarrollo del individuo. Las dos grandes causas de daño cerebral en los adultos se consideran como consecuencia de traumatismos craneoencefálicos y enfermedades vasculares. En cambio, en los ancianos las principales causas suelen ser las enfermedades vasculares y las enfermedades neurodegenerativas (Portellano, 2005). Los daños lesionales producidos por el consumo de alcohol a lo largo de la vida son por tanto daños cerebrales adquiridos, aunque en la práctica clínica sólo se hayan considerado tradicionalmente la encefalitis alcohólica, y las neurodegeneraciones específicas en tractos como cuerpo callos, fimbria-fórnix, como las lesiones específicas generadas por el alcohol.

En base a que una de las consecuencias del daño cerebral adquirido es el deterioro cognitivo, el DSM-5 ha introducido el término de trastorno neurocognitivo y lo ha dividido en tres categorías: delirium, trastorno neurocognitivo leve y trastorno neurocognitivo mayor. Así, introduce el término trastorno neurocognitivo leve (o Deterioro Cognitivo Leve, DCL) para referirse a la fase prodrómica del trastorno neurocognitivo mayor (o

demencia). Los dominios cognitivos que propone para el diagnóstico son: atención compleja, función ejecutiva, aprendizaje y memoria, lenguaje, habilidades perceptuales motoras y el reconocimiento social. Como bien se conoce, el delirium es un estado confusional agudo caracterizado por la alteración de la atención y la conciencia acompañado de cambios en la cognición, que puede producirse por intoxicación a sustancias (ej. delirium tremens producido por el alcohol), además de por otras circunstancias como por medicamentos, afección médica o a etiologías múltiples. No obstante, es importante señalar que el DSM-5 incluye el consumo de sustancias o medicamentos como una de las etiologías desencadenantes del trastorno neurocognitivo leve y mayor (Tabla 6), a parte de la enfermedad de Alzheimer, la demencia frontotemporal, la enfermedad por cuerpos de Lewy, la demencia vascular, el traumatismo craneoencefálico, la infección por VIH, enfermedades por priones, enfermedad de Parkinson, enfermedad de Huntington, otra afección médica, etiologías múltiples o no especificadas (American Psychiatric Association, 2013). En la presente tesis seguiremos los criterios del DSM-5 para trastorno neurocognitivo leve y mayor que se presentan de forma más detallada en la Tabla 4 y 5:

Tabla 4. Criterios para trastorno neurocognitivo leve según el DSM-5.

E. Evidencias de un declive cognitivo modesto comparado con el nivel previo de rendimiento en uno o más dominios cognitivos basados en: a) Quejas sobre la eficiencia cognitiva expresadas por el sujeto, sus allegados o un facultativo. b) Evidencia de declive en el funcionamiento cognitivo, documentado por una evaluación neuropsicológica o clínica equivalente.
F. Desempeño normal de las actividades de la vida diaria, aunque necesita recurrir a estrategias de compensación o de adaptación.

G. Los déficits cognitivos no ocurren exclusivamente en contexto de delirium.
H. Los déficits cognitivos no se explican mejor por otro trastorno mental.

Tabla 5. Criterios para trastorno neurocognitivo mayor según el DSM-5.

A. Evidencias de un declive cognitivo sustancial comparado con el nivel previo de rendimiento en uno o más dominios cognitivos basados en: c) Quejas sobre la eficiencia cognitiva expresadas por el sujeto, sus allegados o un facultativo. d) Evidencia de declive sustancial en el funcionamiento cognitivo, documentado por una evaluación neuropsicológica o clínica equivalente.
B. Los déficits cognitivos interfieren de forma significativa en la autonomía funcional y requiere de asistencia para llevar a cabo actividades de la vida cotidiana.
C. Los déficits cognitivos no ocurren exclusivamente en contexto de delirium.
D. Los déficits cognitivos no se explican mejor por otro trastorno mental.

Tabla 6. Criterios para trastorno neurocognitivo leve o mayor inducido por sustancias o medicamentos según el DSM-5.

A. Se cumplen criterios para el diagnóstico de trastorno neurocognitivo leve o mayor.
B. Los déficits cognitivos no suceden exclusivamente en el transcurso de un delirium y perduran tras la intoxicación y la abstinencia.
C. La duración, la cantidad de consumo y el tipo de sustancia o medicamento son capaces de producir el deterioro neuropsicológico.
D. Los déficits cognitivos coinciden con el periodo de consumo y abstinencia de la sustancia o medicamento y estos se mantienen estables o mejoran tras la abstinencia.
E. Los déficits cognitivos no se explican mejor por otro trastorno mental o afección

médica.

1.3.2. Epidemiología del deterioro cognitivo y la demencia alcohólica

La prevalencia de la demencia alcohólica abarca un amplio rango, desde el 8,27% de cada 100000 hasta el 25,6% de la población, siendo los hombres el género más predominante en los pacientes, que presentan además una proporción de demencia alcohólica de inicio temprano más consistente (Cheng et al., 2017). Aunque para la mayoría de la sociedad, la demencia es un proceso que se equipara con la enfermedad de Alzheimer, un análisis en profundidad revela que esta percepción está fuertemente sesgada en la población general. Así, un estudio en población australiana indicó que el diagnóstico clínico más común de demencia de inicio temprano (≤65 años) fue, en primer lugar, la demencia por alcohol (18,4%), seguido de la enfermedad de Alzheimer (17,7%), la demencia vascular (12,8%) y la demencia froto-temporal (11,3%), siendo la prevalencia mayor para las edades comprendidas entre los 45 y 64 años (Withall et al., 2014). En consonancia con este estudio, Schwarzinger y colaboradores encontraron que el TUA fue el factor más robusto para el incremento del riesgo de demencia en hombres y mujeres, especialmente para aquellas demencias de inicio temprano (Schwarzinger et al., 2018a). Además, se ha demostrado que las diferencias en los hábitos de consumo de alcohol afectan el riesgo de demencia en ausencia de factores genéticos que expliquen estas asociaciones. Así, un estudio retrospectivo exploró el consumo de alcohol en gemelos de mediana edad sobre la incidencia de demencia 43 años más tarde. En gemelos discordantes (si-no demencia), el consumo moderado-elevado de alcohol incrementó un 57% el riesgo de padecer demencia en comparación con aquellos que consumieron cantidades pequeñas de alcohol, y este efecto se acentuó incluso aún más en gemelos monocigóticos, triplicándose el riesgo de demencia. En los gemelos concordantes (si-si demencia) haber tenido un consumo elevado de alcohol fue asociado con el inicio temprano de la demencia (5 años

antes) en comparación con aquellos que consumieron pequeñas cantidades de alcohol (Handing et al., 2014).

1.3.3. Dosis de alcohol y demencia alcohólica

Estudios epidemiológicos longitudinales sugieren una relación en forma de U o J entre el consumo de alcohol y el riesgo de demencia (Figura 7) (Handing et al., 2014; W. Xu et al., 2017), indicando que el consumo leve-moderado de alcohol podría estar asociado con un mejor desempeño cognitivo y un menor declive cognitivo o riesgo de demencia en comparación con aquellos que son abstinentes o bebedores de elevadas cantidades de alcohol (Davis et al., 2014; Kim et al., 2016; Wardzala, 2018; G. Xu et al., 2009). Por ejemplo, en un estudio en población noruega en el que investigaron la incidencia de demencia 27 años después, encontraron que el consumo frecuente (5-10 veces o >10 veces a la semana en 14 días) estuvo asociado con el incremento del riesgo de demencia en comparación con los consumidores infrecuentes (1-4 veces en 14 días) (Langballe et al., 2015). En cuanto a las dosis de alcohol recomendadas, según Xu et al el consumo moderado de alcohol (≤7.5 bebidas/semana o ≤2.5 g/día o ≤2 veces/semana) se asocia con un riesgo reducido de demencia, siendo la dosis de 4 bebidas/semana, 6 g/día o 1 vez/semana la que confiere un menor riesgo, mientras que la dosis ≥23 bebidas/semana o ≥38 g/día se asocia con el mayor riesgo de demencia (W. Xu et al., 2017). Además, en este estudio se observó una reducción del 40% del riesgo de demencia para los consumidores leve-moderados portadores de APOE4. De manera similar, a pesar de que Wardzala no encontró diferencias estadísticas en la incidencia de placas neuríticas (amiloides), sí halló una reducción en la incidencia de nudos neurofibrilares (TAU) en hombres con consumo moderado de alcohol comparados con aquellos que nunca consumen o raramente lo hacen (Wardzala, 2018). Del mismo modo, el consumo de alcohol leve-moderado se ha asociado con un menor riesgo de DCL (G. Xu et al., 2009) y con una menor tasa de progresión a la demencia (Solfrizzi et al., 2007). Sin embargo, a pesar de que los bebedores con un consumo de alcohol leve-

moderado manifiestan mejores puntuaciones en la función cognitiva que los bebedores leves, abstinentes y ex bebedores, los correlatos en neuroimagen señalan a que los hombres con consumo moderado tienen menor volumen cerebral (Davis et al., 2014).

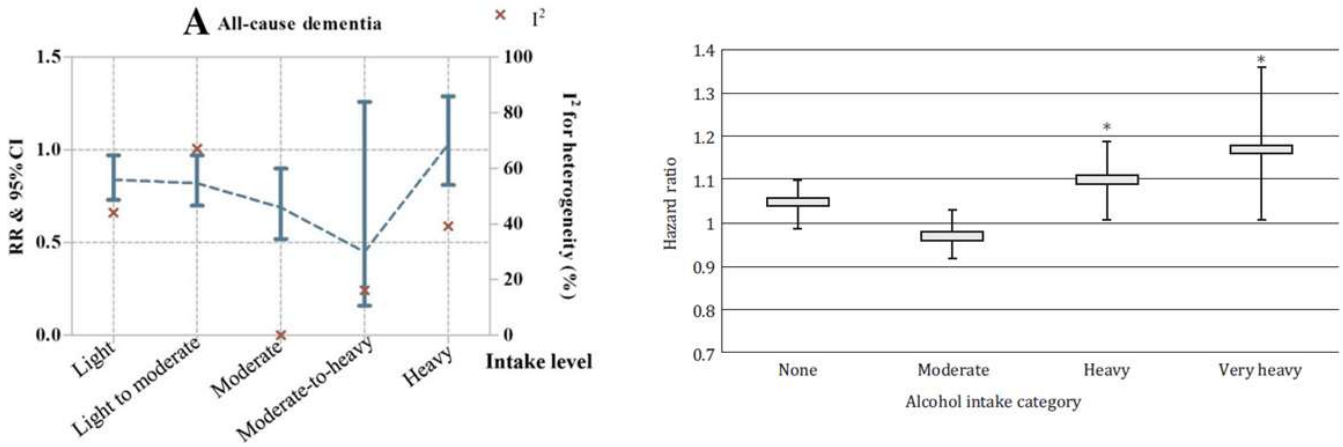


Figura 7. Consumo de alcohol y riesgo de demencia. Figuras extraída de Xu et al (2017) a la izquierda y de Handing et al (2014) a la derecha.

Por otro lado, parece que estos efectos protectores del consumo leve-moderado de alcohol existen solo para determinados tipos de bebidas como el vino y no para otras como la cerveza o los licores, que podrían incrementar el riesgo de demencia (Langballe et al., 2015; W. Xu et al., 2017). Cada gramo adicional de licor se ha asociado a un incremento del 3% en el riesgo de demencia, en cambio, cada gramo adicional de vino se ha relacionado con una disminución del 2%, aunque este efecto se revierte con el consumo de grandes cantidades (Handing et al., 2014).

No obstante, todos estos hallazgos necesitan ser interpretados con cautela debido a dos razones. En primer lugar, las diferencias entre estos estudios hacen difícil su comparación ya que carecen de una definición estandarizada sobre el patrón del consumo (cantidad/bebidas/frecuencia) y se carece de un consenso sobre qué son cantidades "moderadas" alcohol. Asimismo, utilizan distinta metodología, criterios de inclusión, tipos de bebidas y periodos de seguimiento, o no tienen en cuenta otros factores de confusión. A modo de ejemplo, en un estudio longitudinal los resultados indicaron que el mayor riesgo de demencia recayó sobre los pacientes abstinentes de mediana edad, sin embargo, el grupo de

abstinentes se componía de mujeres con un bajo estatus socioeconómico y con una alta prevalencia y riesgo de enfermedad cardiometabólica (Sabia et al., 2018). En segundo lugar, el consumo de alcohol se caracteriza por una alta morbilidad, por lo tanto se podría estar subestimando la incidencia y el riesgo de demencia alcohólica en estos estudios debido a una pérdida significativa de casos (Binder et al., 2017; Ormstad et al., 2016).

1.3.4. Mecanismos de neurotoxicidad del alcohol

Se conoce ampliamente que el consumo abusivo de alcohol puede producir daño neurocognitivo. Sin embargo, uno de los problemas principales que presenta el estudio de la disfunción cognitiva causada por alcohol y la demencia alcohólica es la dificultad para consensuar los criterios diagnósticos y señalar con exactitud el proceso neuropatológico envuelto. Se ha descrito que el deterioro cognitivo y/o la demencia producida por el alcohol se atribuye a factores como la deficiencia nutricional y los efectos neurotóxicos directos del etanol (Hayes et al., 2016; Perry, 2016). Además, existen multitud de variables que podrían explicar o aumentar el riesgo de deterioro cognitivo en pacientes con TUA como diabetes, hipertensión, accidente cerebrovascular, enfermedad cardiovascular, epilepsia, enfermedad hepática, enfermedades infecciosas, fumar tabaco, bajo nivel educativo, etc. (Hayes et al., 2016; Sabia et al., 2018).

En primer lugar, se conoce ampliamente que el déficit de tiamina (vitamina B1) en pacientes con TUA (nutrición deficiente, mala absorción intestinal o fallo hepático) puede producir dos síndromes: la Encefalopatía de Wernicke (aguda) y el Síndrome de Korsakoff (crónica). La Encefalopatía de Wernicke se caracteriza por un cuadro confusional, alteración de la marcha y alteraciones visuales. El Síndrome de Korsakoff es un cuadro determinado por amnesia anterógrada y retrograda severa, desorientación espaciotemporal, apatía, disfunción ejecutiva y ansiedad. Cuando se combinan ambos, síndrome de Wernicke-Korsakoff, conlleva más lesiones cerebrales asociadas a un deterioro cognitivo más severo en comparación con el efecto neurotóxico del alcohol. La tiamina es necesaria

para la respiración celular y el metabolismo de la glucosa, y su deficiencia resulta en estrés oxidativo, excitotoxicidad gutamatérgica e inflamación. Sin embargo, el suplemento de tiamina no es suficiente para revertir los efectos los efectos producidos por su carencia (Mateos-Díaz et al., 2022; Perry, 2016).

No obstante, existen otras vías por las cuales el alcohol puede alcanzar el cerebro y producir neurotoxicidad. En primer lugar, se conoce que el alcohol activa directamente las células inmunes cerebrales (microglía y astrocitos) a través de los receptores *Toll-like* (TLR, específicamente el 4, Alfonco-Loeches et al, 2010) y los receptores NOD-like (NLR) que desencadena en la activación de vías de señalización descendentes rápidas como las proteínas quinasas activadas por mitógenos (MAPKs) y el factor nuclear-kappa B (NF-kB), conduciendo a la generación y liberación de citoquinas, quimioquinas, especies reactivas de oxígeno (ROS) y factores que amplifican la respuesta inflamatoria (ej. complejo multiproteínico del inflamasoma NLRP3) al espacio extracelular alcanzando la neurona y activando vías de muerte celular. De este modo, la inflamación sostenida en el tiempo por un consumo crónico de alcohol resulta en la apoptosis neuronal y en el deterioro de la memoria, la plasticidad neural y la neurogénesis, así como en las enfermedades neurodegenerativas, neuropsiquiatrías y adicciones a drogas (Figura 8) (Alfonso-Loeches et al., 2010; Dwivedi et al., 2018; Montesinos et al., 2016; J. Y. Yang et al., 2014).

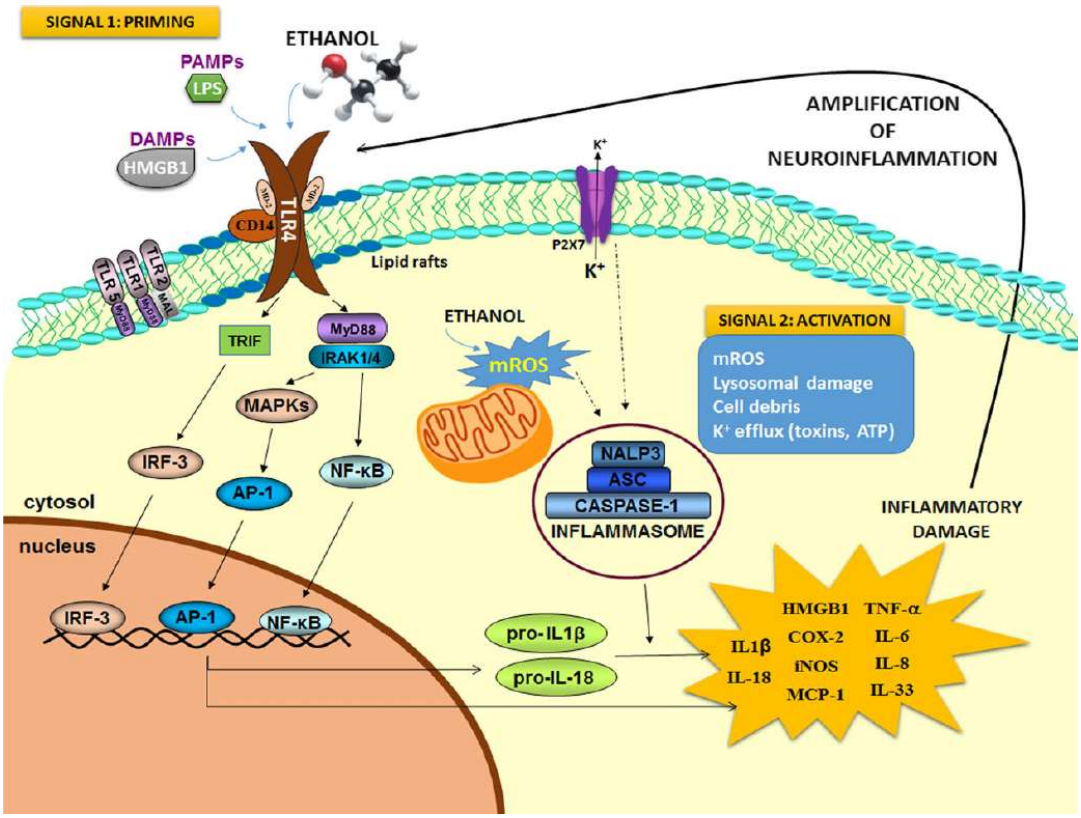


Figura 8. Cascada de señalización inflamatoria resultante de la activación de los receptores TLR4 y el inflammasoma NLRP3 inducida por el consumo de alcohol. Figura extraída de Montesinos et al. (2016).

En segundo lugar, numerosos estudios consideran las mitocondrias una diana importante de la toxicidad del alcohol y sugieren que las alteraciones mitocondriales podrían ser evaluadas como un indicador de la severidad del daño cerebral ya que el consumo de alcohol y la abstinencia por sí solos también inducen alteraciones mitocondriales que repercuten en la biodisponibilidad energética de la neurona e inducen cascadas de señalización apoptóticas (Figura 9). Las mitocondrias son unos orgánulos cuya función es llevar a cabo la respiración celular para producir energía en forma de ATP y la regulación de la apoptosis celular (Tapia-Rojas et al., 2017). Son especialmente abundantes en las terminaciones axonales debido a la alta demanda de energía que requiere el proceso sináptico (Sheng, 2014) y su disfunción se ha asociado con enfermedades neurodegenerativas (Deza-Ponzio, 2018). De este modo, se ha descubierto que el daño

oxidativo producido por el alcohol afecta negativamente a la función bioquímica de las membranas mitocondriales mediante la alteración de la fluidez de la membrana, la permeabilidad de iones y la función de componentes de la cadena de transporte de electrones mitocondrial (Karadayian et al., 2015; Reddy et al., 2013; Tapia-Rojas et al., 2018), los cuales son procesos asociados con el estrés y el daño celular, la apoptosis, la reparación celular y la supervivencia (Tapia-Rojas et al., 2017). Así, la disfunción mitocondrial energética parece influir en la neurotransmisión sináptica ya que se han encontrado reducciones en la LTP (Haorah et al., 2013), junto con la presencia de alteraciones en la memoria, el aprendizaje o la coordinación motora asociadas al alcohol (Jung & Metzger, 2016; Tapia-Rojas et al., 2018). Además, se piensa que el alcohol induce indirectamente alteraciones en las mitocondrias a través de la excitotoxicidad glutamatérgica neuronal que se produce bajo la abstinencia (Frischknecht et al., 2017) que acaba ocasionando la liberación de factores apoptóticos (ej. citocromo C) del interior la mitocondria al citosol de la neurona induciendo la apoptosis celular (Haorah et al., 2013; Wollmuth & Sakmann, 1998; Yun et al., 2014).

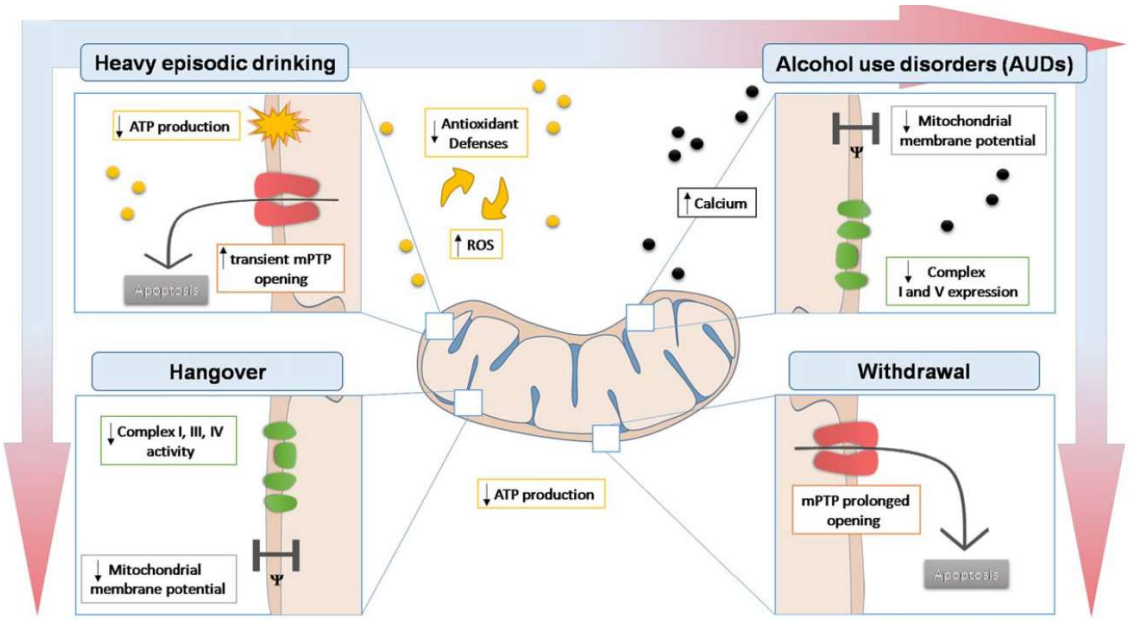


Figura 9. Alteraciones mitocondriales inducidas por el alcohol en el cerebro. Figura extraída de Tapia-Rojas et al. (2017).

Sin embargo, la neuroinflamación inducida por el alcohol también puede originarse a partir de señales inflamatorias que proceden de tejidos periféricos como el intestino, el hígado o el páncreas y pueden llegar hasta el cerebro a través de la alteración de la barrera intestinal, que permite el paso de productos bacterianos inflamatorios, y de la barrera hematoencefálica, que permite que todas estas moléculas proinflamatorias alcancen a las neuronas (Banks, 2015). Por ejemplo, el alcohol puede inducir alteraciones de las uniones estrechas en el epitelio de la mucosa intestinal, lo cual aumenta la permeabilidad a lipopolisacárido (LPS, componente de la membrana externa de las bacterias Gram negativas) y permite su ingreso al torrente sanguíneo. Después, el LPS circulante llega al hígado y estimula los TLR4 en las células de Kupffer para dar como resultado el aumento de citoquinas y quimioquinas proinflamatorias que pueden cruzar la barrera hematoencefálica y activar el sistema inmune cerebral a través de la microglía y los astrocitos (Figura 10) (Montesinos et al., 2016).

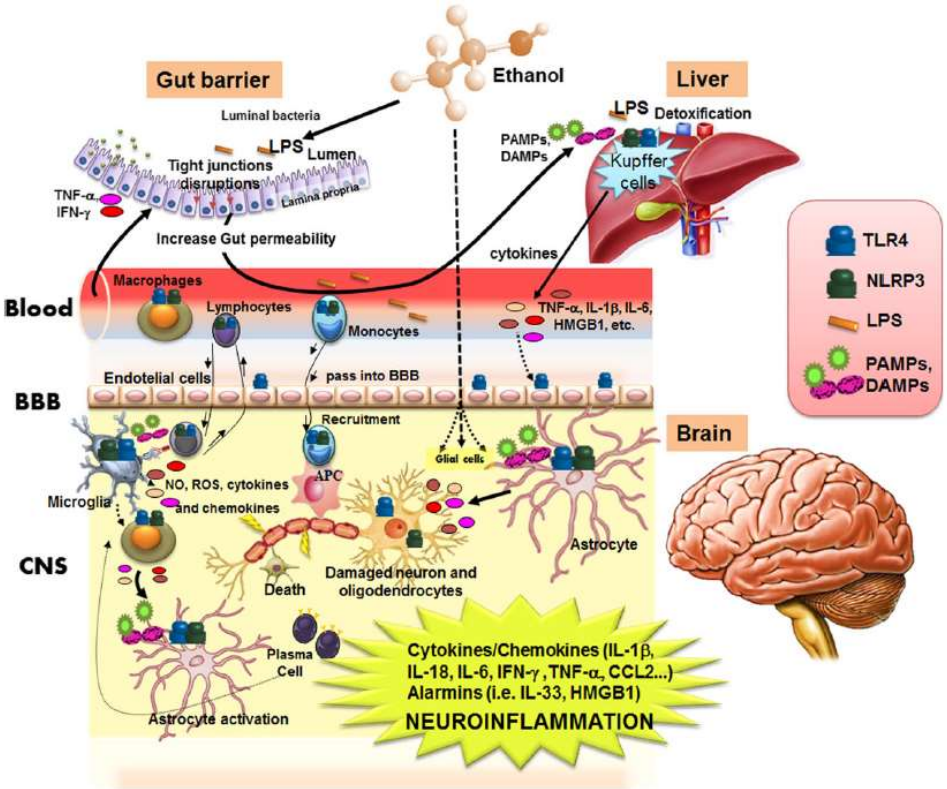


Figura 10. Neuroinflamación producida indirectamente por el consumo de alcohol en tejidos periféricos. Figura extraída de Montesinos et al. (2016).

Además, es importante señalar que el metabolismo del alcohol no supone la desintoxicación del organismo, ya que los aldehídos son mucho más tóxicos que los alcoholes originales y son considerados como una sustancia mutagénica y carcinogénica, por lo que el rol del etanol en el cáncer y en las enfermedades neurodegenerativas podría estar subestimándose (Deza-Ponzio, 2018; Pohanka, 2016).

1.4. Función cognitiva asociada al consumo de alcohol

1.4.1. Evaluación neuropsicológica

La evaluación neuropsicológica nos permite conocer el estado de las funciones cognitivas superiores, el comportamiento del individuo y la integridad cerebral especialmente cuando se ha producido algún tipo de daño cerebral. De este modo, el cerebro trata de reorganizar, compensar o sustituir sus funciones cuando este se produce (Portellano, 2005).

El objetivo general de la evaluación neuropsicológica es contribuir a la mejora del funcionamiento cognitivo, la conducta, la adaptación y la calidad de vida de las personas que han sufrido daño cerebral. Con el objetivo de contribuir a su diagnóstico, es necesario la utilización de pruebas psicométricas o clínicas para conocer el nivel de eficiencia cognitiva del sujeto en relación con las estructuras encefálicas subyacentes. De este modo, se podrán conocer los puntos fuertes y débiles de cada paciente que servirán de apoyo para la rehabilitación, el diseño de programas de intervención neuropsicológica y la valoración de su progreso a lo largo del tratamiento (Bruna, O., Roig, T., Puyuelo, M., Junqué, C., & Ruano, 2011). A continuación se presentan las principales funciones cognitivas y una breve definición de las mismas (Pérez, J. A. P., & Alba, 2014):

Velocidad de procesamiento y atención

El modelo de Sohlberg y Mateer (1989) describe un sistema atencional jerárquico y cada nivel requiere el correcto funcionamiento del nivel anterior asumiendo que cada

componente es más complejo que el que le precede (Sohlberg, M. M., & Mateer, 1987, 1989). Está conformado por cinco niveles cuyos dos primeros están relacionados con el proceso atencional básico propio del lóbulo parietal, mientras que los tres últimos están relacionados con un proceso atencional más complejo ligado a las funciones ejecutivas del lóbulo prefrontal. Los niveles son: 1) *arousal* (capacidad de estar despierto y de mantener la alerta), 2) atención focalizada (habilidad para enfocar la atención a un estímulo), 3) atención sostenida (capacidad para mantener una respuesta de forma consistente durante un período de tiempo prolongado), 4) atención alternante (capacidad que permite cambiar el foco de atención entre tareas que implica requerimientos cognitivos diferentes), 5) atención dividida (capacidad para atender a dos cosas al mismo tiempo) (Fig. 14).

Memoria

La memoria es la capacidad para codificar la información a partir de la extracción de un significado, almacenar o retener la información codificada de forma persistente, y recuperar o reconocer la información almacenada (Tulving, 1972). Según Atkinson y Shiffrin (1968) el proceso mnésico se estructura de la siguiente forma: 1) registros sensoriales (es el procesamiento perceptivo que retiene la información sensorial durante un corto periodo de tiempo), 2) la memoria a corto plazo/memoria operativa (retiene la información recibida por un periodo de tiempo (segundos) antes de almacenarla o desecharla y la procesa activamente de forma consciente y controlada, su longitud es de 6,5 ± 2 piezas de información), y 2) la memoria a largo plazo (almacenamiento permanente e ilimitado de la información) (Atkinson, R. C., & Shiffrin, 1968). A su vez, la memoria a corto plazo se divide en la memoria declarativa y en la procedimental (Squire, L. R., Knowlton, B., & Musen, 1993). La memoria procedimental es el conocimiento implícito de cómo hacer las cosas que se adquiere con la práctica y, por lo tanto, tiene un componente automático. En cambio, la memoria declarativa se refiere a la adquisición de hechos o datos accesibles a la conciencia y se divide en: 1) la memoria episódica (vivencias personales,

situaciones y sucesos que se experimentan a lo largo de la vida y que están asociados al contexto espaciotemporal), y 2) la memoria semántica (conocimientos de carácter general sobre el mundo y nosotros mismos que no depende del contexto espaciotemporal).

Memoria de trabajo

Es un sistema que almacena y manipula información para el razonamiento, el aprendizaje y la comprensión. Utiliza información a partir de la memoria a corto plazo y largo plazo para resolver problemas. El modelo de Baddley y Hitch (1974) describe la memoria de trabajo como un sistema complejo de almacenamiento de la información verbal y visual en forma de multicomponentes (Baddeley, A. D., & Hitch, 1974). Estos autores proponen los siguientes módulos: agenda visoespacial (mantiene activa la información visoespacial), bucle fonológico (mantiene activa la información verbal), sistema ejecutivo central (sistema atencional interno que opera con distintos tipos de información) y el buffer episódico (mecanismo por el que traemos información de la memoria a largo plazo para utilizarla a corto plazo).

Funciones ejecutivas

Las funciones ejecutivas son procesos supra modales de alto nivel que nos permiten realizar actividades mentales complejas, planificar, organizar, guiar, revisar, regularizar y evaluar el comportamiento necesario para adaptarnos al entorno y alcanzar metas (Damasio, A. R., & Anderson, 2003).

Se denominan funciones ejecutivas, en plural, debido a que encontramos diferentes tipos de procesos: 1) actualización (adquisición, inserción y manipulación de nuevas informaciones), inhibición (supresión de la información no relevante o de las respuestas automáticas), flexibilidad (capacidad para adaptar las respuestas a nuevas contingencias, generando nuevos patrones de conducta al tiempo que se inhiben aquellas respuestas que son inadecuadas), planificación (capacidad para identificar y organizar las

secuencias necesarias para conseguir un objetivo), toma de decisiones (habilidad para seleccionar la opción más ventajosa en un contexto dado, entre un repertorio de varias alternativas disponibles), fluencia (capacidad para emitir la respuesta más eficiente con el menor coste posible). A estos procesos de regulación cognitiva se les han denominado funciones "*cold*", no obstante, las funciones ejecutivas también poseen un componente de regulación emocional llamado funciones "*hot*" (control emocional, autoconsciencia, conciencia ética, empatía, capacidad para la interacción social, regulación del estado de ánimo, etc.). Las funciones "*cold*" están ligadas a la corteza prefrontal dorsolateral y ventrolateral, mientras que las funciones "*hot*" están muy vinculadas a la corteza orbitofrontal (Pérez, J. A. P., & Alba, 2014).

Praxias

Las praxias son las habilidades motoras adquiridas que nos permiten realizar movimientos voluntarios previamente aprendidos. Dentro de las praxias se pueden clasificar varios tipos: 1) la praxia ideomotora (ejecución de movimientos simples de manera voluntaria), 2) la praxia ideatoria (realización de secuencias motoras complejas necesarios para utilizar objetos correctamente), y 3) la praxia visoconstructiva (capacidad para reproducir los movimientos necesarios para organizar una serie de elementos en el espacio y conseguir figuras bidimensionales o tridimensionales. También existen otras praxias como la de la marcha, la bucofacial, del habla, la óptica, la callosa, la cruzada, etc. Las bases neurales de las praxias son el córtex frontal, que se encuentra más activo con el aprendizaje de nuevas habilidades motoras, y los ganglios basales donde se produce la automatización del movimiento (Pérez, J. A. P., & Alba, 2014).

Gnosias

Las agnosias se definen como el déficit en el reconocimiento de estímulos que no puede ser atribuido a defectos sensoriales. Es decir, se produce la incapacidad para

reconocer estímulos familiares y atribuirles el significado correcto. Así encontramos una gran variedad de agnosias visuales (prosopagnosia, simultagnosia, agnosia cromática, agnosia visual para objetos, agnosia visual para dibujos, acinetopsia), agnosias auditivas (amusia, agnosia de sonidos no verbales, agnosia verbal pura), agnosias somestésicas (astereognosia, agnosia táctil, barognosia, autotopagnosia, agnosia digital, planotopocinesia), agnosia olfatoria (anosmia, normosmia, hiperosmia, parosmia, cacosmia). Incluso existen agnosias para las enfermedades como la anosognosia (falta de conciencia sobre un déficit cognitivo o motor), asomatognosia (incapacidad para reconocer, integrar y diferenciar partes del sistema corporal), somatoparafrenia (atribución del miembro paralizado a otra persona), analgoagnosia (incapacidad para identificar el dolor), etc (Portellano, 2005).

1.4.2. Alteraciones neuropsicológicas derivadas del consumo del alcohol

El deterioro de la función ejecutiva parece ser una de las competencias más afectadas en los trastornos por uso de sustancias (TUS). Varios estudios han observado un pobre desempeño en fluidez y flexibilidad mental, cambio atencional, interferencia, impulsividad, razonamiento analógico y toma de decisiones en comparación con los sujetos controles (Fernández-Serrano et al., 2010; Warren et al., 2017), siendo la memoria de trabajo el componente más deteriorado en los pacientes policonsumidores (Fernández-Serrano, Pérez-García, Perales, et al., 2010). Los daños en diferentes sistemas del córtex prefrontal, como la corteza orbitofrontal, la corteza dorsolateral y el cíngulo anterior, explicarían el comportamiento apático, desinhibido y la disfunción cognitiva que se asocian a los dominios de la vida cotidiana en los que estos pacientes suelen manifestar problemas (Verdejo-García et al., 2006; Verdejo-García et al., 2006).

Específicamente, los pacientes TUA muestran alteraciones en la velocidad de procesamiento, la atención sostenida, la memoria y el aprendizaje, así como en las funciones ejecutivas a nivel global, siendo la cognición visoespacial el componente más

afectado (Sachdeva et al., 2016). Los pacientes muestran un pobre control cognitivo, baja flexibilidad cognitiva, desinhibición, falta de planificación y de estrategias para la resolución de problemas y dificultades en memoria de trabajo visoespacial (Horton et al., 2015; Kopera et al., 2012; Sabbe et al., 2012). Además, padecen alteraciones en el procesamiento emocional y en la cognición social (Freeman et al., 2018; Le Berre et al., 2017).

Por otro lado, variables como el inicio, la duración, la gravedad del consumo pueden influir en el deterioro cognitivo de los pacientes TUS, aunque aún no se ha establecido un consenso sobre cómo repercuten. El inicio temprano del consumo de alcohol parece ser un factor de riesgo para un funcionamiento neuropsicológico posterior más pobre (Nguyen-Louie et al., 2017). El consumo temprano de alcohol predice peores resultados en los dominios de velocidad psicomotora y atención visual, siendo el consumo semanal de alcohol al inicio un potente predictor de la disfunción de la memoria de trabajo y una mayor desinhibición en el futuro (Nguyen-Louie et al., 2017). Del mismo modo, un pobre control inhibitorio/impulsividad en la adolescencia podría ser un buen predictor de mal uso del alcohol en el futuro (Whelan et al., 2014). Sin embargo, otros estudios apoyan que el deterioro de la función cognitiva por el consumo prolongado de alcohol es independiente de la edad del inicio del consumo. Personas mayores que consumieron alcohol desde los veinte años presentaron similares habilidades cognitivas en comparación con aquellos que empezaron a consumir después de los cuarenta, aunque ambos presentaron déficits neuropsicológicos en comparación con el grupo control (Kist et al., 2014). Estas diferencias en los resultados podrían explicarse disociando los efectos neurocognitivos a corto y largo plazo que surgen del consumo elevado de alcohol. En esta línea, los resultados de Woods et al sugieren que los efectos neurocognitivos a corto plazo podrían surgir como resultado de la gravedad del consumo actual, que pueden ser exacerbados por la edad del individuo (consumidores de elevadas cantidades de alcohol ≥40 años exhiben mayores déficits

cognitivos en comparación con los consumidores de elevadas cantidades de alcohol jóvenes y con los adultos abstinentes); mientras que los efectos neurocognitivos a largo plazo se podrían deber únicamente al historial de dependencia de alcohol independientemente de la edad del individuo (Woods et al., 2016). Es importante destacar que el deterioro cognitivo de los pacientes TUA puede suavizarse a partir del año de abstinencia, pero la reversibilidad del deterioro es limitada. A pesar de existir evidencia sobre la recuperación parcial de ciertas funciones cognitivas (Kopera et al., 2012; Ros-Cucurull et al., 2018), los déficits en otras funciones pueden permanecer de forma estable en la abstinencia (Romero-Martínez et al., 2018; Sabbe et al., 2012; Sachdeva et al., 2016).

Además, el deterioro cognitivo puede comprometer los esfuerzos para iniciar y mantener la abstinencia al impactar en la efectividad del tratamiento (Le Berre et al., 2017). Parece que cuanto más perjudicadas se encuentren las funciones ejecutivas mayor es la probabilidad experimentar una recaída. En un estudio analizaron la asociación entre los déficits cognitivos y las tasas de recaídas en personas bajo 6 meses de abstinencia y encontraron que el predictor más fuerte para la recaída fue la interacción entre el número de previas desintoxicaciones y los déficits en respuesta inhibitoria (Czapla et al., 2016). Parece que una mayor vulnerabilidad al deterioro de la memoria de trabajo inducido por el alcohol puede repercutir en la capacidad para moderar su uso (Lechner et al., 2016) y el entrenamiento cognitivo podría reducir el consumo de bebidas en estos pacientes (Khemiri et al., 2019).

1.4.3. Factores protectores: la reserva cognitiva

La Reserva Cognitiva (RC) es un concepto destinado a explicar la discrepancia frecuente entre el nivel medido de patología cerebral de un individuo y el rendimiento cognitivo esperado. Es particularmente importante en el contexto del envejecimiento y la demencia, pero su aplicabilidad se extiende a todas las formas de daño cerebral (Barulli & Stern, 2013). De hecho, existe una fuerte evidencia de que una alta RC es capaz de proteger

desde los procesos normales de envejecimiento (Cadar et al., 2016) hasta la aparición de DCL (Mazzeo et al., 2019) , enfermedad de Alzheimer (Bessi et al., 2018; Colangeli et al., 2016), enfermedad de Parkinson (Hindle et al., 2014), esclerosis múltiple (Fenu et al., 2018), daño cerebral relacionado con trauma, accidente cerebrovascular o tumor (MacPherson et al., 2017; Mathias & Wheaton, 2015); así como trastornos psiquiátricos como depresión, bipolaridad, ansiedad o psicosis (Forcada et al., 2015; Koenen et al., 2009). A pesar de que no existe un modelo generalizado sobre RC, esta parece ser el resultado de la inteligencia innata o cociente intelectual premórbido, los años de escolarización, el nivel ocupacional, el estado socioeconómico, las actividades de ocio, la actividad física y la interacción social (Clare et al., 2017; Mathias & Wheaton, 2015; M. Yuan et al., 2018).

No obstante, es importante señalar que la RC tiene un rol dual en el declive cognitivo y la demencia (Figura 11): una alta RC resulta ser un factor protector para la progresión de declive cognitivo subjetivo a DCL, retrasando su progresión 9 años (Liu et al., 2013; Mazzeo et al., 2019); mientras que una elevada RC puede ser un factor de riesgo para la progresión de DCL a demencia, ya que anticipan el diagnóstico de Alzheimer 4 años antes aunque solo en los portadores de APOE4 (Mazzeo et al., 2019). Es decir, los resultados sugieren que los mecanismos mediante los cuales la RC media en la relación entre patología y función cognitiva se debe más al retraso del comienzo de la sintomatología en lugar de reducir la tasa de deterioro cognitivo. A través de los individuos que progresan a DCL y tienen altos niveles de biomarcadores de Alzheimer, una mayor RC estuvo asociada con un mejor desempeño cognitivo antes del comienzo de los síntomas clínicos, y con un declive cognitivo más rápido después del inicio de los síntomas de DCL (Rusmaully et al., 2017; Soldan et al., 2017).

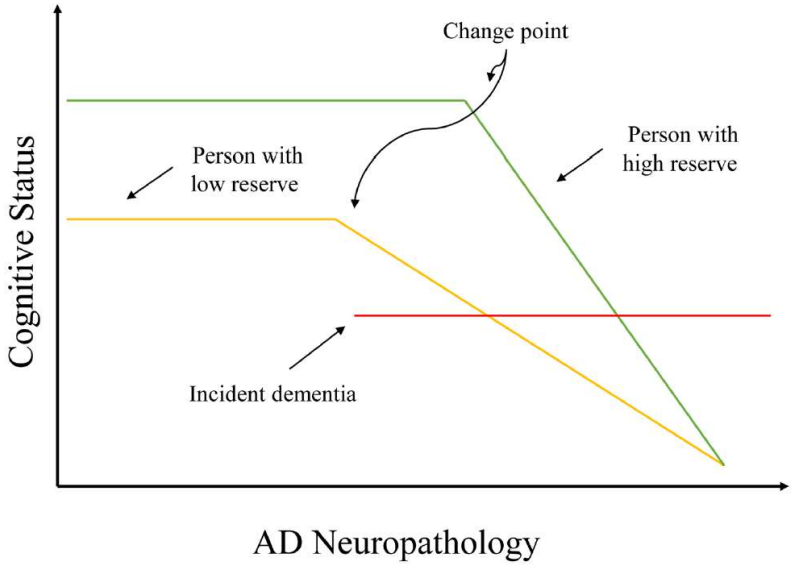


Figura 11. Mecanismo por el cual la RC media en la patología AD y en sus manifestaciones clínicas. La cantidad de patología necesaria antes de que la función cognitiva se vea afectada es mayor con una elevada RC, retrasando así la aparición de la enfermedad. Una vez que comienza el deterioro cognitivo, es más rápido en la persona con mayor RC. Figura extraída de Barulli & Stern (2013).

Recientemente se ha incorporado el constructo de RC al campo de las adicciones. Los estudio sugieren que la RC puede jugar un papel preventivo en el inicio del consumo de drogas, el desarrollo de la adicción y la gravedad de los problemas relacionados con las sustancias, así como con una mejor respuesta al tratamiento (Cutuli et al., 2019). De este modo, los jóvenes que abandonan la educación secundaria o la universidad tienen un mayor riesgo de desarrollar abuso de alcohol y sustancias ilícitas en la vida adulta en comparación con aquellos que completaron estos logros académicos (Crum et al., 1998; Levine et al., 2017). Asimismo, la evidencia actual sugiere que la RC en adicciones contribuye a mejorar el rendimiento neuropsicológico y se ha relacionado con menores indicadores de trastornos neurocognitivos (Aldebarán Toledo-Fernández et al., 2019). Por ejemplo, un estudio longitudinal señala que un nivel educativo inferior a la escuela secundaria y una baja ocupación laboral se asocia con un mayor riesgo de demencia en pacientes alcohólicos (Sabia et al., 2018).

1.5. Marcadores biológicos de alcohol y deterioro cognitivo

La investigación sobre los sustratos que median el reforzamiento positivo de las drogas ha dominado el campo de las adicciones a lo largo de los años. El incremento de la señalización dopaminérgica se ha propuesto como el sustrato neuronal crítico para explicar los efectos producidos por las drogas. Sin embargo, los procesos adictivos envuelven adaptaciones en numerosos sistemas neuroquímicos a los que no se les ha otorgado tanta relevancia, así como a factores moduladores de la arquitectura y función cerebral, como los factores de crecimiento o las citoquinas proinflamatorias, que podrían estar contribuyendo a la aparición de disfunción cognitiva y enfermedad mental.

1.5.1. Factores de crecimiento

Los factores de crecimiento son un conjunto de proteínas que juegan un papel principal en la estimulación de la proliferación, supervivencia y diferenciación celular. Entre la familia de factores de crecimiento se encuentran las neurotrofinas (NGF, NT-3, NT-4 y BDNF), el factor de crecimiento nervioso (FCN), el factor de crecimiento endotelial vascular (VEGFA), y otros factores de crecimiento asociados a tipos celulares específicos como el CNTF, GDNF o el PDGF (Barde, 1990; Ferrara et al., 1991). En la presente tesis nos hemos enfocado en tres de ellos, BDNF, 3-NT y VEGFA, basándonos en estudios previos de nuestro laboratorio (García-Marchena et al., 2017; Silva-Peña et al., 2019).

1.5.1.1. Factor neurotrófico derivado del cerebro

El Factor Neurotrófico Derivado del Cerebro (BDNF) es un miembro de la familia de las neurotrofinas, que incluye el factor de crecimiento nervioso (NGF), neurotrofina 3 (NT3), y neurotrofina 4 (NT4). Las funciones más importantes que se atribuyen al BDNF incluyen procesos de desarrollo, regulación de neuro-, glio- y sinaptogénesis, neuroprotección, y el control de la potenciación a largo plazo (LTP) y la depresión a largo plazo (LTD), que son cambios duraderos en la fuerza sináptica que median en los

mecanismos de memoria y cognición (Kowiański et al., 2018; Panja D, 2014; Waterhouse EG, 2009).

Existen distintos tipos de BDNF según su maduración que interactúan con distintos receptores produciendo vías de señalización distintas. De este modo, el pro-dominio (polimorfismo Val66Met) interacciona con el receptor sortilin y la neurotrofina inmadura pro-BDNF interacciona tanto con p75NTR como con el receptor sortilin. El complejo de unión pro-BDNF/p75NTR/sortilin inicia cascadas de señalización apoptóticas (c-Jun amino terminal kinasa-JNK), vías que regulan la proyección y dirección axonal (miembro A de la familia del gen homologo Ras- RhoA), y vías de supervivencia neuronal y mantenimiento del número adecuado de neuronas durante el desarrollo cerebral (factor nuclear kappa-NF-kB). En cambio, la neurotrofina madura m-BDNF interacciona con el receptor TrKB, que pertenece a la familia de tirosina quinasas receptoras de neurotrofinas, y su unión activa cascadas de señalización neuroprotectoras como la vía inositol fosfatidico 3-kinasa PI3K/Akt, la vía proteína quinasas activadas por mitógenos (MAPK), la vía C-gamma fosfolipasa (PLC-gamma) y la vía guanosine trifosfato hidrolasa (GTP-asa) (Figura 12) (Kowiański et al., 2018). Por ende, el BDNF comprende funciones que pueden parecer contradictorias. Participa tanto en la LTD a través del isomorfismo pro-BDNF como en la LTP a través del m-BDNF. El primer isomorfismo se relaciona con la etapa del desarrollo cerebral (proceso de poda sináptica y apoptosis) y en procesos patológicos (lesión cerebral), mientras que el segundo participa en la etapa adulta manteniendo la neurona y procurando el crecimiento neuronal a través de la sinaptogénesis, neurogénesis y gliogénesis, arborización axodendrítica, etc. Por lo tanto, altos niveles de pro-BDNF y bajas concentraciones de m-BDNF conllevan a la eliminación neuronal (Kowiański et al., 2018).

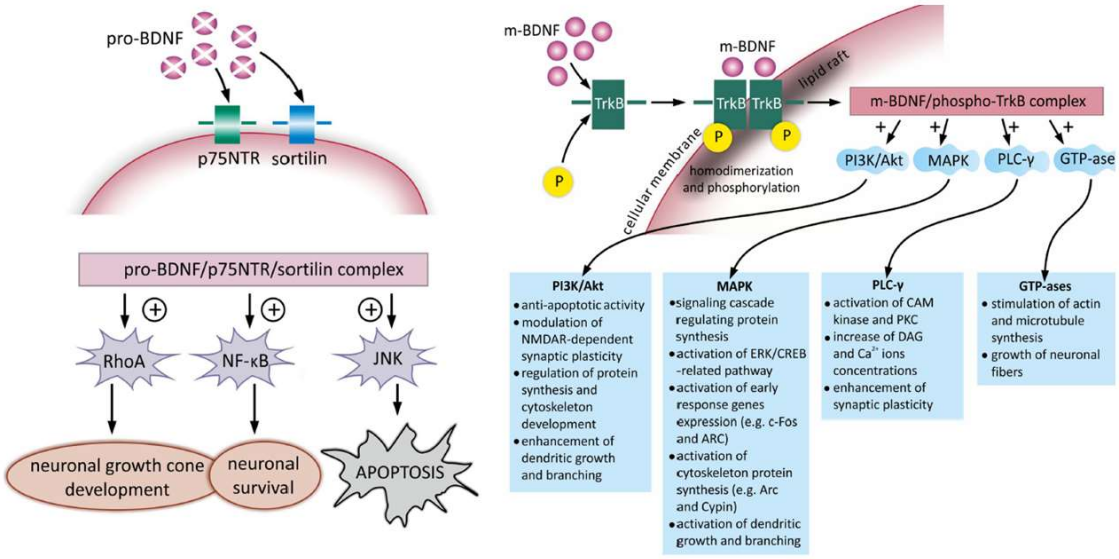


Figura 12. Cascadas de señalización en función de la maduración del BDNF. A la izquierda, el pro-BDNF (neurotrofina inmadura) puede desencadenar la apoptosis neuronal. A la derecha, el m-BDNF (neurotrofina madura) puede desencadenar vías neuroprotectoras. Figura extraída de Kowiański et al (2018).

En relación con lo anterior, parece haber un gran consenso en la literatura sobre la asociación entre el déficit en los niveles de BDNF plasmático y el declive cognitivo en pacientes con trastorno neuropsiquiátrico como depresión, esquizofrenia, trastorno bipolar, epilepsia, enfermedad de Parkinson, DCL y enfermedad de Alzheimer (Siuda et al., 2017; Wang et al., 2016; B. Yang et al., 2017). Asimismo, nuestro grupo de investigación ha asociado recientemente el BDNF con el deterioro cognitivo de pacientes con TUA durante la abstinencia (Silva-Peña et al., 2019). A pesar de que el BDNF no es un biomarcador clasificatorio al no ser capaz de distinguir entre múltiples trastornos neurodegenerativos, puede ser considerado un buen predictor del grado de deterioro y progresión del mismo (Buchman et al., 2016; D. Woolley et al., 2012; Küster et al., 2017). Por último, el BDNF parece ser un candidato para describir la base neurobiológica de la RC ya que la actividad física, las interacciones sociales, la estimulación cognitiva, un alto nivel educativo y experiencias de un ambiente enriquecedor se han asociado con aumentos en sus niveles y un

menor riesgo de demencia (Beeri & Sonnen, 2016; Håkansson et al., 2017; Heisz et al., 2017; Küster et al., 2017; Siuda et al., 2017).

1.5.1.2. Neurotrofina 3

La Neurotrofina 3 (NT-3) es otra proteína que pertenece a la familia de las neurotrofinas y se ha relacionado con la neurogénesis, el desarrollo y mantenimiento de neuritas, así como con el establecimiento de conexiones sinápticas (Gómez-Palacio-Schjetnan, A., & Escobar, 2013). Cuando la NT-3 se une al receptor TRkC, que pertenece a la familia de tirosina quinasas receptoras de neurotrofinas, desencadena cascadas de señalización que regulan la supervivencia celular, la proliferación y la motilidad a través de la fosfolipasa Cγ1 (PLCG1), a vía fosfatidilinositol 3 kinasa (PI3K) y RAS-MAPK (Figura 13). En ausencia de NT-3, el receptor TRkC promueve la apoptosis desencadenando la muerte celular dependientes de BAX y CASP9. No obstante, actividad proapoptótica de TRkC está implicada en el correcto desarrollo del sistema nervioso (Health, 2022). Además, del mismo modo que ocurre con el BDNF, el pro-NT-3 podría estar implicado en acciones proapoptóticas neuronales mediante los receptores p75 y sortilin (Yano, H., Torkin, R., Martin, L. A., Chao, M. V., & Teng, 2009).

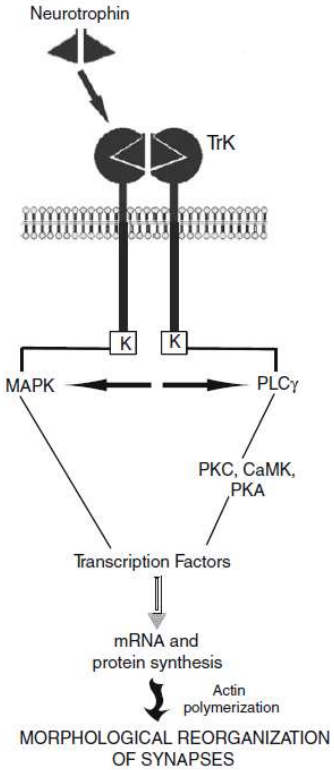


Figura 13. Cascadas de señalización mediante la activación TRkC inducida por NT-3. Figura extraída de Gómez-Palacio-Schjetnan, A., & Escobar (2013).

Por otra parte, el rol de la NT-3 se ha relacionado más con el desarollo del SNC que en los procesos patológicos de envejecimiento (Murase, K., Igarashi, K., & Hayashi, 1994). De este modo, en un estudio preclínico se encontró una expresión desregulada de NT-3 tanto en el cerebro neonatal como en el hipocampo adulto, lo que podría explicar los déficits cognitivos del síndrome de Down (Pollonini et al., 2008). Sin embargo, si que podría estar involucrado en ciertos procesos compensatorios cuando se produce daño cerebral (Aili, L. I., Zhou, G., Fan, E., & Liu, 2006). En esta línea, nuestro grupo ha descrito concentraciones plasmáticas menores de NT-3 en pacientes con TUA que en controles (Silva-Peña et al., 2019).

1.5.1.3. Factor de crecimiento endotelial vascular

El factor de crecimiento endotelial vascular (VEGFA) es una proteína comúnmente conocida por su rol en la angiogénesis y en la permeabilidad vascular. A través del receptor VEGFR2, VEGFA es capaz de activar múltiples vías de señalización que resultan en: 1) la angiogénesis, a través de la proteína quinasa regulada por señal extracelular (ERK), fosfatidilinositol- 3-quinasa (PI3K)/Akt, SRC-kinasas, quinasa de adhesión focal (FAK) y la familia de proteínas G, 2) la permeabilidad vascular, a través de SRC-kinasas, proteína G Rac, fosfatasas y óxido nítrico endotelial (eNOS); y 3) la inflamación que, aunque VEGFA no es una citoquina inflamatoria, puede activar las cascadas de señalización factor nuclear potenciador de las cadenas ligeras kappa de las células B activadas (NF-KB) y el factor nuclear de células T activadas (NFAT) (aunque aún se desconoce este papel) (Claesson-Welsh, L., & Welsh, 2013) (Figura 14).

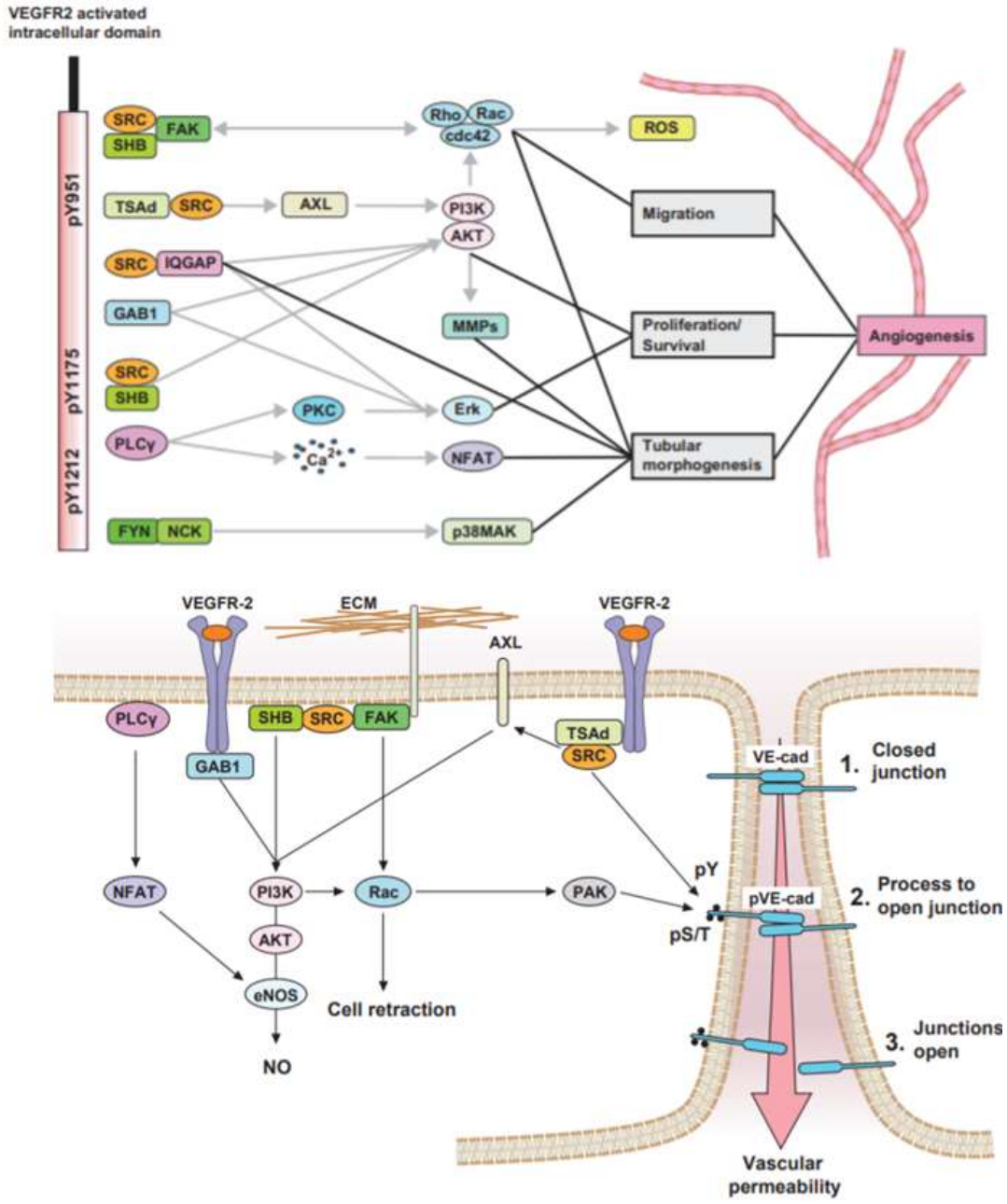


Figura 14. Cascadas de señalización de la angiogénesis y permeabilidad vascular inducida por VEGFA y su receptor VEGFR2. Figuras extraídas de Claesson-Welsh, L., & Welsh (2013).

Además, este factor de crecimiento juega un papel fundamental en el desarrollo y el sistema nervioso adulto ya que interviene en la extensión y la complejidad de la microvasculatura que suministra los nutrientes y el oxígeno necesarios en el cerebro. De este modo, los efectos de VEGFA sobre el sistema nervioso se han relacionado la neuroprotección, la neurogénesis y la plasticidad sináptica en a través de la estimulación de las células madre neuronales y salvaguardando la integridad de la barrera hematoencefálica

(Lange et al., 2016). Por lo tanto, los cambios en las concentraciones de VEGFA podrían afectar a la función y supervivencia de las neuronas al no proveer suficientes nutrientes o producir hipoxia, lo cual se ha relacionado con el deterioro de la función cognitiva (Klintsova et al., 2013). Además, VEGFA es un potente factor angiogénico y vasodilatador liberado en condiciones hipóxicas y estresantes a través de la óxido nítrico sintasa endotelial (Bates, 2010; Tammela et al., 2005). Por lo tanto, niveles alterados de VEGFA se han relacionado con varios trastornos neurodegenerativos y neurológicos, como Alzheimer, demencia vascular e ictus (Chiappelli, 2006; Lange et al., 2016; Tarkowski, 2002).

1.5.2. Mecanismos neuroinflamatorios: quimioquinas

Las quimioquinas (o citoquinas quimiotácticas) son pequeñas proteínas con un bajo peso molecular (7-15kDa) producidas por células inmunes que se encargan de la migración celular y la coordinación inmunológica. De este modo, el sistema inmune puede detectar lesiones tisulares o patógenos mediante un gradiente de citoquinas que terminan activando la actividad inflamatoria (Palomino, D. C. T., & Marti, 2015). Algunas quimioquinas se consideran proinflamatorias y su liberación puede inducirse durante una respuesta inmunitaria en un sitio de infección o lesión, mientras que otras se consideran homeostáticas y están involucradas en el control de la migración celular durante el desarrollo o mantenimiento de tejidos (Fig. 22). Además, las citoquinas intervienen en la regulación del desarrollo celular, su supervivencia y la regeneración del SNC (Comerford, I., & McColl, 2011).

Podemos diferenciar tres familias de quimioquinas basadas en el primer residuo de cisteína: 1) la familia CC (o β-quimioquinas) tienen dos cisteínas cerca de su extremo amino terminal y estimulan principalmente a los monocitos, pero también a los basófilos, eosinófilos, linfocitos T y células asesinas naturales (NK); 2) la familia CXC (o α-quimioquinas) tienen un aminoácido intermedio entre las dos primeras cisteínas y estimulan principalmente la quimiotaxis de neutrófilos (Palomino, D. C. T., & Marti, 2015); y 3) la

familia CX3C tienen tres aminoácidos entre las dos cisteínas y solo se ha descubierto que pertenezca a esta familia la fractalquina o CX3CL1. Los neutrófilos juegan un papel fundamental en el control de infecciones debido a su capacidad de fagocitar microorganismos y por la liberación de otros mediadores quimiotácticos que reclutan otros leucocitos a los tejidos dañados (Comerford, I., & McColl, 2011).

Por otro lado, los receptores de quimioquinas son receptores acoplados a proteína G que abarcan siete dominios transmembrana y se expresan principalmente en la superficie de los leucocitos. La interacción entre las quimioquinas con el receptor provoca su activación y se produje la división del complejo de proteína G en las subunidades Gα y Gβγ (Figura 15). Estos segundos mensajeros juegan un papel fundamental en la activación de diversas cascadas de transducción de señales, lo que lleva a la quimiotaxis de los leucocitos y así como a otras respuestas inmunes (Comerford, I., & McColl, 2011). De este modo, en el presente trabajo nos hemos enfocado en la exploración de cinco quimioquinas distintas:

- **CCL2/MCP-1**: es la mejor reconocido por sus acciones quimiotácticas y activadoras sobre monocitos/macrófagos, linfocitos T y células dendríticas. Además, es un inductor débil de la expresión de citoquinas en los monocitos y, en altas concentraciones, puede conducir a la producción de especies reactivas de oxígeno (Zapata, 2000). La CCL2 también puede dirigir la migración y diferenciación de células madre/progenitoras neurales en el neurodesarrollo (Stuart & Baune, 2014). Los niveles de CCL2 en el líquido cefalorraquídeo se ha correlacionado con un deterioro cognitivo más rápido en pacientes prodrómicos con Alzheimer (Westin et al., 2012).
- **CCL3/MIP-1α**: es producida por macrófagos, células dendríticas y linfocitos, y activa neutrófilos, eosinófilos y basófilos, e inducen la activación de otras citoquinas proinflamatorias (IL-1, IL-6 y TNF-α) (Ali, S., Palmer, A. C., Banerjee, B., Fritchley, S. J., & Kirby, 2000). Se conoce que es capaz de controlar

la migración de células en diferentes regiones del sistema nervioso aunque su rol en neuropatología sigue siendo desconocido (Stuart & Baune, 2014).

- **CCL11/Eotaxina**: es un quimioatrayente local responsable del reclutamiento de eosinófilos y, por tanto, está muy ligado a la inflamación alérgica y el asma (Jose et al., 1994). MIP-1α se ha propuesto como la "quimioquina de envejecimiento cerebral acelerado" ya que está asociada con el deterioro cognitivo relacionado con la edad así como en procesos neurodegenerativos (Ivanovska et al., 2020; Sirivichayakul et al., 2019; Teixeira et al., 2018).
- **CXCL12/SDF-1**: presenta una fuerte actividad quimiotáctica en relación con los monocitos/macrófagos y linfocitos, y es capaz de controlar la migración de células en diferentes regiones del sistema nervioso en desarrollo (Janowski, 2009; Tiveron, M. C., & Cremer, 2008).
- **CX3CL1/Fractalkina**: es una quimioquina atípica, ya que es el único miembro conocido de la familia CX3C, y tiene un papel importante en el desarrollo de numerosas enfermedades inflamatorias, incluida la aterosclerosis. Es un factor quimiotáctico único que existe tanto en forma unida a la membrana como soluble. Cuando se encuentra en la membrana media en la captura de leucocitos, mientras que en su estado soluble funciona como un potente quimioatrayente de células diana (Palomino, D. C. T., & Marti, 2015). Además, desarrolla un papel fundamental en la intercomunicación neuronal-microglial (Sokolowski et al., 2014).

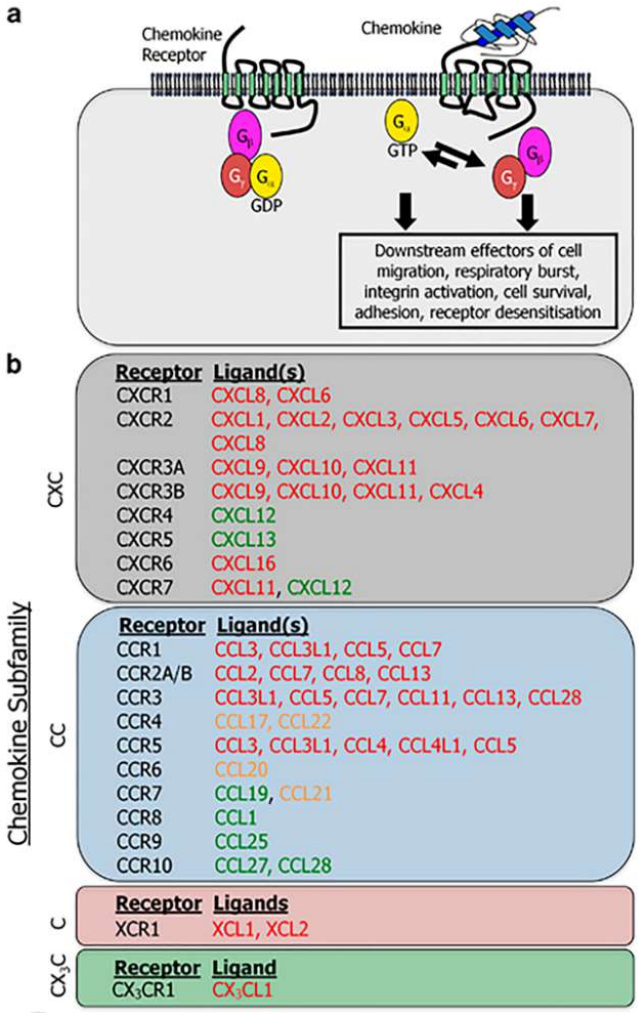


Figura 15. Familia de quimioquinas y sus receptores. Las quimioquinas en rojo son proinflamatorias, las verdes son homeostáticas. Figura extraída de Comerford, I. & McColl (2011).

De este modo, se ha descrito una descompensación de quimioquinas en plasma, suero y líquido cefalorraquídeo en varias enfermedades psiquiátricas y neurodegenerativas, como DCL, enfermedad de Alzheimer, demencia vascular, enfermedad de Parkinson, esquizofrenia, trastorno bipolar y depresión mayor (Hu et al., 2019; Italiani et al., 2018; Ivanovska et al., 2020; Magalhães et al., 2018; Nordengen et al., 2019). De este modo, se conoce que las quimioquinas son componentes importantes del sistema neuroinmune que contribuyen a la actividad neuronal, la función neuroendocrina, el desarrollo cerebral, la plasticidad sináptica, la función cognitiva y los circuitos del estado de ánimo en la adicción a las drogas (Cui, C., Shurtleff, D., & Harris, 2014; Lacagnina et al., 2017). Además, es importante mencionar que una respuesta inflamatoria exacerbada en el cerebro puede no

proporcionar suficiente BDNF para los procesos neuroplásticos necesarios (González-Reimers et al., 2014; Patterson, 2015).

Como ya se ha mencionado anteriormente, se piensa que la neuroinflamación podría estar causada por el efecto directo del alcohol sobre el cerebro o por el efecto del alcohol sobre la permeabilidad del intestino (Banks, 2015). Se conoce ampliamente que el consumo problemático de alcohol ocasiona una condición sistémica proinflamatoria ya que impacta en el sistema inmunológico e induce una regulación positiva de citoquinas y quimioquinas (González-Reimers et al., 2014; Jung & Metzger, 2016). Además, varios estudios han informado que los comportamientos relacionados con el alcohol se interrumpen cuando se altera la red inmunitaria innata (Pascual M, Balino P, Aragon CM, 2015; Yen et al., 2017). Por ejemplo, un estudio ha demostrado que la eliminación del gen CCR2, MCP-1 o MIP-1α reduce los efectos motivacionales del consumo de alcohol en ratones (Blednov et al., 2005).

1.5.3. Marcadores de daño neuronal: neurofilamentos

Los neurofilamentos (NfLs) son filamentos intermedios (~10 nm de diámetro), que se encuentran entre los filamentos de la actina (6 nm de diámetro) y la miosina (15 nm de diámetro), y son un constituyente importante del andamiaje del citoesqueleto de las neuronas tanto del SNC como del periférico. Los NfLs están compuestos por cuatro polímeros diferentes: 1) de cadena ligera (NfL, ≈70 kDa), 2) de cadena media (NfLM, ≈150 kDa), 3) NLs de cadena pesada (NfLH, ≈200 kDa), más 4) una α -internexina en el SNC (≈66 kDa) y una periferina en el sistema nervioso periférico (≈57 kDa). Todas estas subunidades comparten una estructura común compuesta por un dominio de cabeza globular amino-terminal, un dominio de barra helicoidal α central y una cola carboxi-terminal. Sin embargo, los NfM y los NfH tienen además en la cola carboxi-terminal repeticiones de serina-prolina-lisina fuertemente fosforiladas (Khalil et al., 2018; A. Yuan et al., 2017).

Los NfLs son un indicador de daño neuroaxonal, que es el sustrato patológico de la discapacidad permanente en varios, sino todos, los trastornos neurológicos agudos y crónicos (A. Yuan et al., 2017). Cuando se daña un axón, los NfLs se liberan al espacio extracelular que pasan al líquido cefalorraquídeo y, en concentraciones más bajas, a la sangre. Por lo tanto, los NfLs parecen ser un biomarcador candidato muy prometedor para reflejar los daños en el tejido cerebral de enfermedades de índole neurológico, y que podría permitir el seguimiento de la enfermedad, así como los efectos de la rehabilitación y los tratamientos farmacológicos (Khalil et al., 2018; Verde et al., 2021). De este modo, un estudio de validación multicéntrica ha establecido unos valores de referencia para los controles sanos y las principales enfermedades neurológicas a partir de los estudios realizados en dos cohortes (Figura 16): la King's College London (n=805) y la Lund del estudio Swedish BioFINDER (n=1.464) (Ashton et al., 2021).

Además, aunque los NfLs pueden no ser específicos para distinguir trastornos cognitivos como β-amiloide y Tau en la enfermedad de Alzheimer (Koychev et al., 2021), han demostrado ser útiles para diferenciar ciertos trastornos neurológicos que podrían diagnosticarse erróneamente [ej. disociar el trastorno parkinsoniano atípico de la enfermedad de Parkinson (86 %-95 %)] (Ashton et al., 2021; Montoliu-Gaya et al., 2021), así como para evaluar si un proceso neurodegenerativo está en curso o es consecuencia de trastornos psiquiátricos [ej. distinguir la demencia frontotemporal de la depresión (98 %)] (Ashton et al., 2021; Davy et al., 2021).

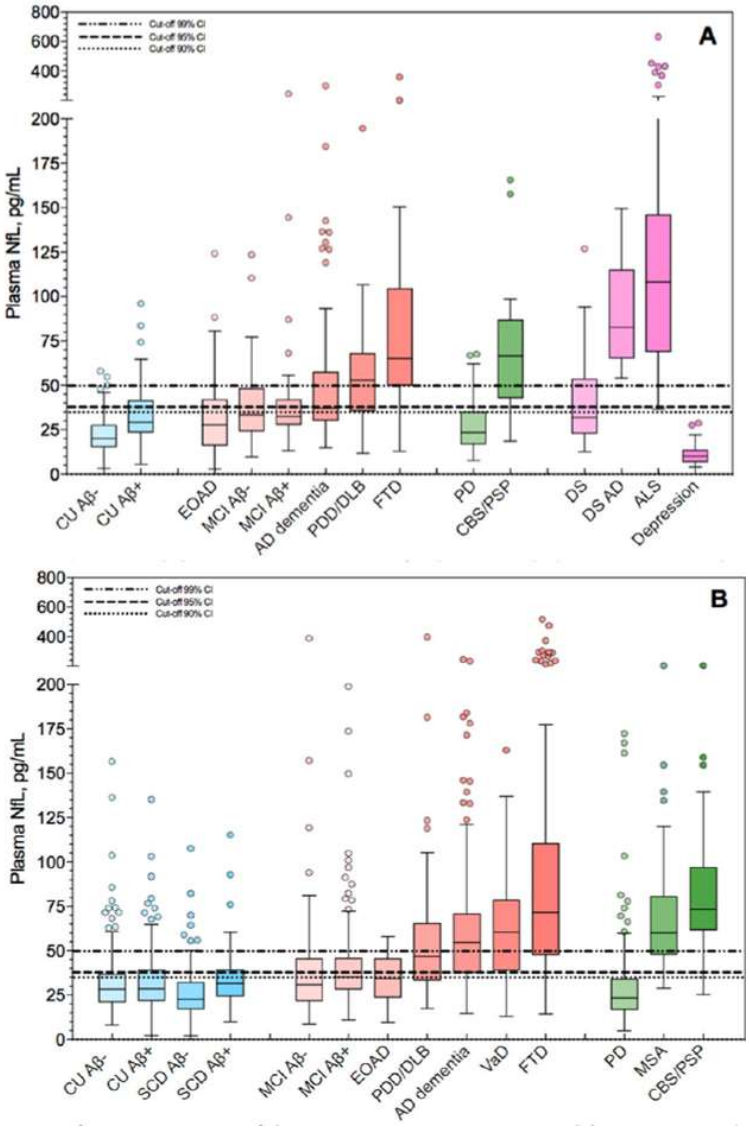


Figura 16. Concentraciones plasmáticas de NfL de diferentes trastornos neurológicos y controles de las cohortes King's College London y Lund. Abreviaciones: CU = sin deterioro cognitivo, VaD = demencia vascular, FTD = demencia frontotemporal, PD = enfermedad de Parkinson, PDD = demencia de la enfermedad de Parkinson, AD = enfermedad de Alzheimer, EOAD = enfermedad de Alzheimer de inicio temprano, ALS = esclerosis amiotrófica lateral, CBS = síndrome corticobasal, PSP = parálisis supranuclear progresiva, DBL =demencia con cuerpos de Lewi, DS = síndrome de Down, DSAD = síndrome de Down con enfermedad de Alzheimer, MSA = sistema de atrofia múltiple, MCI = DCL, SCD = deterioro cognitivo subjetivo. Figura extraída de Ashton et al. (2021).

La investigación en NfLs es relativamente reciente y carece de estudios científicos en pacientes con abuso de sustancias. Por ahora, únicamente se encuentra el artículo de Karoly et al (2021) en el cual no se observó una asociación directa entre el NfLs en plasma y el

consumo excesivo de alcohol; aunque sí encontraron una correlación negativa significativa entre el grosor de la materia gris y los NfLs circulantes (Karoly et al., 2021). No obstante, este estudio se realizó en individuos sanos consumidores de altas cantidades de alcohol y no en pacientes con un diagnóstico de adicción a sustancias. Por ello, en la presente tesis nos parece de gran relevancia evaluar los niveles plasmáticos de NfLs en pacientes con TUA para explorar si el consumo patológico de alcohol produce realmente una neurodegeneración cerebral y una disminución crónica de las capacidades cognitivas. Además, los NfLs parecen un candidato aún más atractivo para evaluar el daño cerebral asociado al alcohol, ya que se ha demostrado que son más sensibles discriminando enfermedades neurodegenerativas en personas jóvenes (<65 años) que en personas mayores (>65 años) (Ashton et al., 2021).

2. Hipótesis y objetivos

Como ya se ha desarrollado en la introducción, es evidente que la adicción al alcohol está estrechamente relacionada con la aparición de deterioro cognitivo leve y su posterior evolución a demencia: el consumo de alcohol puede desencadenar o adelantar la aparición del declive cognitivo y la neurodegeneración, sea cual sea la causa primaria que la origine. Además, ambas enfermedades comparten alteraciones neurobiológicas, como el impacto sobre los factores de crecimiento y el sistema inmune. El presente trabajo está orientado a demostrar que el consumo problemático de alcohol a lo largo de la vida ocasiona un deterioro cognitivo de base neuroinflamatoria y trófica que puede evolucionar a una demencia de inicio temprano. El daño cerebral progresivo se produciría a través de la alteración de los factores neurotróficos como el BDNF, las alteraciones de la barrera hematoencefálica producidas por una liberación anormal del factor de crecimiento vascular endotelial y la desregulación de la liberación de quimioquinas. Todos estos procesos culminarían con el deterioro de la función cognitiva, los mecanismos de plasticidad, la neurogénesis y, en definitiva, con la muerte neuronal. La actividad tóxica del alcohol sobre

las neuronas (proapoptótica o de necrosis neuronal) en los pacientes con TUA se pretende demostrar a través de la cuantificación de los niveles NfLs que es un biomarcador fiable de neurodegeneración que se ha establecido recientemente. Para llevar a cabo este trabajo, se fijaron las siguientes hipótesis y objetivos:

2.1. Hipótesis

I. En pacientes con trastorno por uso de alcohol que demandan tratamiento ambulatorio, existe una elevada prevalencia de deterioro cognitivo asociado al consumo, que se extiende a varios dominios cognitivos (función ejecutiva y memoria).

II. El nivel de reserva cognitiva del paciente, medido a través del nivel educativo conseguido, la reserva cognitiva juega un papel protector o de riesgo en el desarrollo de la enfermedad adictiva y en la aparición de deterioro cognitivo.

III. En los pacientes con trastorno por uso de alcohol que demandan tratamiento ambulatorio, los niveles plasmáticos de los factores tróficos BDNF, Neurotrofina-3 y VEGFA están alterados y estas alteraciones están relacionadas con la RC y el deterioro cognitivo, por lo que actúan como biomarcadores de prevención y patología.

IV. En los pacientes con trastorno por uso de alcohol que demandan tratamiento ambulatorio, las alteraciones plasmáticas presentes en las concentraciones circulantes de algunas quimioquinas proinflamatorias están relacionadas con la presencia de deterioro cognitivo, por lo que podrían servir como biomarcadores de dicha patología.

V. En los pacientes con trastorno por uso de alcohol que demandan tratamiento ambulatorio, se encuentran alteraciones en los niveles de NfL similares a los procesos neurodegenerativos que se observan en otras demencias.

VI. Es posible establecer un conjunto discreto de biomarcadores de deterioro cognitivo/demencia en pacientes con trastorno por uso de alcohol con validez diagnóstica, pronóstica y de respuesta a tratamiento.

2.2. Objetivos

I. Reclutar una muestra de pacientes en tratamiento por trastorno por uso de alcohol y realizar la evaluación psiquiátrica y neuropsicológica para analizar la prevalencia y severidad del deterioro cognitivo, así como la presencia de comorbilidad psiquiátrica. A través de la recopilación de esta información podremos:

   a) Comprobar si el deterioro cognitivo de estos pacientes es consecuencia directa del consumo de alcohol o se debe a la presencia de comorbilidad psiquiátrica.
   b) Analizar si el deterioro cognitivo es producido por el alcohol o por la comorbilidad de abuso de otras sustancias como la cocaína o el cannabis.
   c) Analizar si el deterioro cognitivo es de carácter disejecutivo, mnésico o frontotemporal (ambos).
   d) Analizar si el deterioro cognitivo de estos pacientes es reversible o es de carácter crónico.

II. Obtener información biológica de estos pacientes a partir de la extracción de sangre periférica para realizar la determinación de los factores de crecimiento y quimioquinas en plasma, cuyos niveles se compararán con una población control de referencia. De este modo podremos:

   a) Comprobar que existen diferencias en las concentraciones plasmáticas entre pacientes (sujetos con TUA que demandan tratamiento) y controles.
   b) Constatar que existen alteraciones en las concentraciones plasmáticas de estos biomarcadores entre pacientes con y sin deterioro cognitivo, estableciendo cuales podrían ser potenciales biomarcadores de deterioro cognitivo con utilidad clínica.

III. Comprobar si la reserva cognitiva de los pacientes, medida a través del nivel educativo, influye en las variables asociadas con el consumo de sustancias (inicio del consumo, inicio del trastorno por uso de sustancias, severidad de la adicción, longitud de la abstinencia y duración del Trastorno por uso de sustancias), así como el desarrollo de deterioro cognitivo en los pacientes con trastorno por uso de alcohol específicamente.

IV. Caracterizar si existen alguna asociación entre los potenciales biomarcadores plasmáticos y la reserva cognitiva, en función del nivel educativo alcanzado (primaria, secundaria, universidad).

V. Demostrar a través de la cuantificación de NfLs que el deterioro cognitivo observado en los pacientes TUA es fruto de la neurodegeneración y, por tanto, nos encontramos ante la aparición de una toxicidad neuronal producida directa o indirectamente (vía neuroinflamación o alteraciones tróficas) por el alcohol, y que abocaría a la aparición de un deterioro cognitivo o una demencia de inicio temprano. Para ello se compararán los pacientes con trastorno por uso de alcohol con un grupo poblacional con demencia y un grupo control.

3. Metodología

3.1. Diseño general de los estudios clínicos

El marco metodológico de la presente tesis se basa en el estudio descriptivo-observacional de carácter transversal de una población de pacientes que acuden a tratamiento ambulatorio en dispositivos asistenciales hospitalarios por el diagnóstico de TUS. Los pacientes son de ambos sexos y se estudian en abstinencia mantenida. La exploración y caracterización de esta población se llevó a cabo mediante cuatro estudios clínicos de los cuales, en los dos últimos, los participantes se compararon con una muestra de sujetos sanos pertenecientes al grupo control. Los datos clínicos de los estudios realizados en la población con abuso de sustancias se recogen a partir de entrevistas de

evaluación psiquiátrica y pruebas de evaluación neuropsicológica. Los datos fisiológicos se obtienen a través de determinaciones plasmática de analitos que podrían servir como potenciales marcadores biológicos relacionados con los TUS y el deterioro cognitivo (Figura 17). A parte de los estudios descriptivos, también se incluye un estudio de revisión sobre reserva cognitiva en adicciones. Los estudios de la presente tesis son los siguientes:

- Estudio 1: "*Evaluation of neurotrophic factors and education level as predictors of cognitive decline in alcohol use disorder*" publicado en Scientific Reports.
- Estudio 2: "Influence of gender and education on cocaine users in an outpatient cohort in Spain" publicado en Scientific Reports.
- Estudio 3: "*VEGFA as potential biomarker of neuroinflammation and frontal cognitive impairment in patients with alcohol use disorder*" publicado en Biomedicines.
- Estudio 4: "*Structural brain damage related to alcohol use disorder and cognitive impairment: alterations in Neurofilaments light protein and Brain Neurotrophic Factor"* en revisión editorial.
- Revisión: "*Cognitive reserve in substance use disorders and potential biological markers"* en revisión editorial.

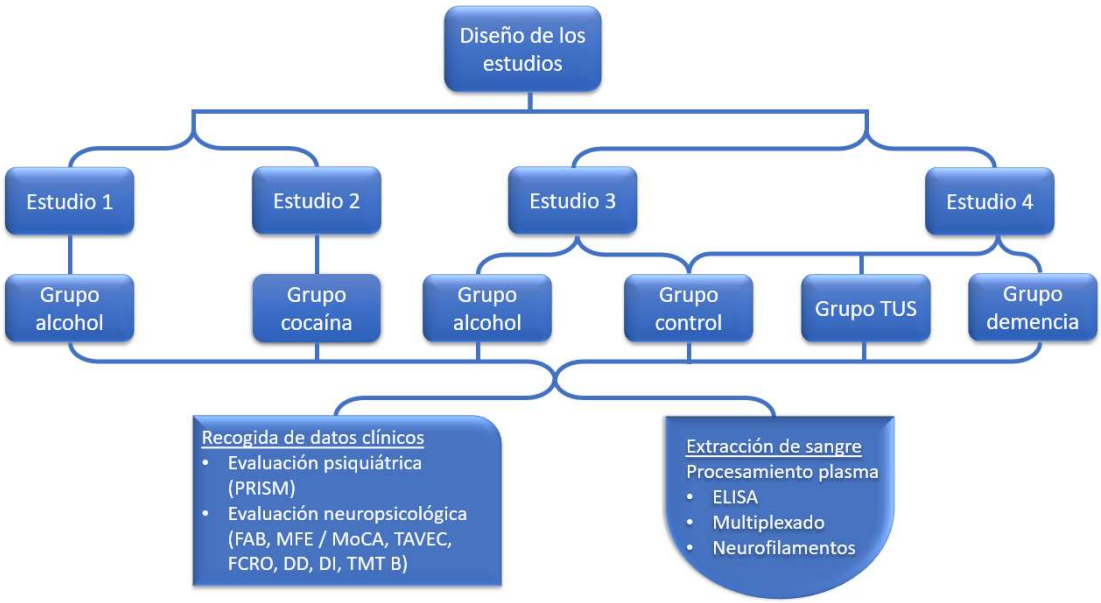


Figura 17. Diseño de los estudios de la tesis doctoral.

3.1.1. Descripción de la población de estudio

La población participante en este trabajo estaba formada por sujetos de ambos sexos de raza blanca caucásica. Los pacientes fueron reclutados desde los servicios de psiquiatría del Hospital 12 de octubre de Madrid y de Centros Provinciales de Drogadicciones de Málaga en función de la existencia de un historial de TUS. Los Estudios 1 y 2 de la presente tesis no se compararon con un grupo control ya que 1) en el Estudio 1 las diferencias en las concentraciones de BDNF y NT-3 entre el grupo alcohol y el grupo control ya se habían descrito en estudios anteriores de nuestro grupo de investigación, y 2) el Estudio 2 es un estudio estrictamente descriptivo sobre las variables sociodemográficas y psiquiátricas de la población que acude a tratamiento ambulatorio por consumo de cocaína.

Sin embargo, en los Estudios 3 y 4 los pacientes TUS se compararon con un grupo control con el objetivo de investigar las diferencias en las concentraciones plasmáticas de quimioquinas, VEGFA y NfLs. Así, el grupo control se compuso de sujetos sanos que fueron incluidos desde la base de datos del Biobanco Nacional de ADN (Salamanca,

España). Los controles fueron seleccionados para obtener una población equilibrada en cuanto a la edad, índice de masa corporal (IMC) y ratio de sexo con el grupo TUS.

Además, en el Estudio 4 se añadió un tercer grupo de pacientes con Demencia compuesto por pacientes derivados de los servicios de neurología del Hospital Regional Universitario de Málaga y el Hospital Civil de Málaga con el fin de tener un control poblacional de sujetos con signos de déficits cognitivos (queja subjetiva de memoria) o diagnóstico de demencia en los cuales está bien establecido el aumento de NfLs tanto en plasma como en líquido cefalorraquídeo. Por cuestiones obvias, el grupo demencia no pudo controlarse por edad, aunque se obtuvo una población equilibrada en cuanto a IMC y ratio de sexo con el grupo TUS y el grupo control.

3.1.2. Criterios de participación

El reclutamiento de la población para los estudios que componen esta tesis se basó en el cumplimiento de unos criterios de participación. Los participantes fueron mayores de edad, con una edad comprendida entre 18 a 66 años, y habían accedido a participar de forma voluntaria y anónima a través de la firma de los consentimientos informados pertinentes. Existen algunas diferencias entre los criterios de inclusión y exclusión entre los diferentes grupos y estudios que se especifican que se especifican a continuación:

Criterios de inclusión del grupo TUS:

Los participantes incluidos tener un diagnóstico de TUS y encontrarse bajo tratamiento ambulatorio en un programa de deshabituación guiada. La sustancia principal por la que habían acudido a tratamiento debía haber sido principalmente el alcohol, a pesar de que podía haber otras sustancias de consumo o problemática asociada. El diagnóstico del TUS fue determinado mediante una entrevista personal de evaluación psiquiátrica individualizada basada en criterios DSM-IV-TR y corroborados por el historial médico del paciente. Los pacientes debían estar en abstinencia al menos dos semanas previas a la

extracción de muestra biológica. La monitorización de la abstinencia se realizaba en enfermería las primeras semanas con la toma ambulatoria de la medicación en presencia de los sanitarios, y a través de un alcoholímetro antes de realizar la analítica para comprobar que el contenido de alcohol en el aire espirado fuese 0 g/L en el momento de la extracción.

Un criterio de inclusión exclusivo del Estudio 2 fue el diagnóstico de Trastorno por Uso de Cocaína como sustancia principal, debido a que nuestro objetivo fue investigar si la influencia de la reserva cognitiva (operativizada a través del nivel educativo) sobre las variables relacionadas con la adicción que fueron descubiertas en el Estudio 1 en pacientes TUA se podría extrapolar a otras drogas de abuso frecuente como la cocaína.

Criterios de inclusión del grupo control

Los criterios de inclusión que se tuvieron en cuenta para la participación de los sujetos controles fueron los siguientes: no haber tenido diagnóstico de trastornos por uso de sustancias a lo largo de su vida ni historial de comorbilidad psiquiátrica concomitante, no haber padecido enfermedad médica grave y que estuviesen cognitivamente sanos. Además, se escogieron en función del estudio para que estuviesen pareados en edad, IMC y sexo con el grupo de pacientes TUS.

Criterios de inclusión del grupo demencia:

Los criterios de inclusión que se tuvieron en cuenta para los pacientes de demencias fueron: tener una edad >60 años, encontrarse bajo tratamiento neurológico y haber obtenido una puntuación <24 en el *Mini-Mental State Examination* (MMSE). Además, su participación debía ser voluntaria, anónima y consensuada a través de la firma de los pertinentes consentimientos informados que, en casos de avanzada enfermedad, eran firmados por un tutor legal.

Criterios de exclusión del grupo TUS y el grupo demencias:

Los criterios de exclusión que se tuvieron en cuenta para la participación en los distintos estudios para ambos grupos fueron los siguientes:

- La ausencia de enfermedades infecciosas comprobado a través de pruebas de rápida detección en plasma para VIH, hepatitis B y C. En cada caso positivo, la muestra de sangre se eliminó siguiendo un protocolo de seguridad de laboratorio para minimizar los riesgos de infección del personal sanitario e investigador.
- Alteraciones cognitivas y psiquiátricas incapacitantes para completar la entrevista psiquiátrica y neuropsicológica.
- El rechazo de la participación por parte del paciente.

Otros criterios de exclusión específicos del grupo TUS fueron mujeres embarazadas y antecedentes de enfermedad inflamatoria o cáncer para evitar la alteración de parámetros biológicos.

3.2. Aspectos éticos de los estudios clínicos

Todos los participantes fueron informados mediante una descripción completa de los estudios antes de la realización de la entrevista clínica y de la extracción de la sangre. La presente tesis se enmarca en los proyectos impulsados por la Red de Trastornos Adictivos [RTA], entidad financiada por el Instituto de Salud Carlos III (ISCIII) perteneciente al Ministerio de Ciencia e Innovación de España. Los aspectos éticos del proyecto central fueron aprobados por el Comité de Ética e Investigación Clínica del Hospital Regional Universitario de Málaga, respetando los principios éticos para la investigación médica en seres humanos adoptados en el Declaración de Helsinki de la Asociación Médica Mundial (64a Asamblea General de la AMM, Fortaleza, Brasil, 2013). La autorización del comité ético se encuentra en el Anexo.

3.3. Evaluación clínica

Un miembro del equipo clínico colaboró en cada centro de tratamiento ambulatorio para la captación de los participantes. Esta persona se encargó de informar sobre la existencia del estudio e invitar a pacientes que habían solicitado tratamiento en algún momento para participar en los estudios. Si cumplían los criterios de inclusión establecidos,

los pacientes eran derivados al equipo de psicólogos clínicos que se encargaban de citar a los pacientes y de desplazarse por los distintos centros de tratamiento ambulatorio de la provincia de Málaga para la evaluación de los pacientes. En los estudios clínicos se realizó la evaluación minuciosa de cada paciente de estudio mediante herramientas de evaluación clínica, realizada por un psicólogo con formación especializada y acreditada en evaluación psicopatológica y neuropsicológica, con el fin de detectar posibles patologías psiquiátricas adicionales al TUS, así como signos de deterioro cognitivo. La evaluación clínica se realizaba por la mañana y podía durar entre dos y cuatro horas (Tabla 7). Finalmente, cada entrevista se registró en una base de datos anonimizada, especialmente diseñada para el estudio.

3.3.1. Evaluación psiquiátrica

Todos los participantes del estudio fueron evaluados mediante la *Psychiatric Research Interview for Substance and Mental Diseases* (PRISM) para recopilar datos sociodemográficos y evaluar los trastornos psiquiátricos comórbidos de acuerdo con los criterios del DSM-IV-TR. Este instrumento es una entrevista semiestructurada, con cualidades psicométricas en términos de fiabilidad test-re-test, fiabilidad entre distintos evaluadores y validez para el diagnóstico de trastornos psiquiátricos en consumidores de sustancias de abuso (coeficiente Kappa entre 0,66 y 1,00) (Hasin et al., 2013; Torrens et al., 2004). El primer módulo de preguntas evalúa la historia del consumo de sustancias y el segundo módulo evalúa veinte trastornos del Eje I y dos del Eje II que son más prevalentes en esta población. Una de las características más importantes de este instrumento es que permite diferenciar los trastornos mentales primarios de los inducidos por sustancias y de los efectos esperados de la intoxicación y de la abstinencia (Araos et al., 2014, 2015; Garcia-Marchena, Araos, et al., 2017; Garcia-Marchena, Pavon, et al., 2017).

3.3.2. Evaluación neuropsicológica

Las evaluaciones neuropsicológicas para el grupo TUS se realizaron de forma individual y fueron realizadas por psicólogos capacitados en evaluación neuropsicológica. En un principio, con la finalidad de valorar el estado cognitivo de los pacientes nos decantamos por la prueba *Frontal Assessment Battery* (FAB), para medir la función ejecutiva, y el *Memory Failure Everyday 30* (MFE-30) para evaluar la memoria. Estas pruebas son de administración rápida, cuentan con una alta validez y eficacia, y engloban las áreas cognitivas más afectadas en los TUS (1ª batería neuropsicológica, Tabla 7). No obstante, cuando obtuvimos signos de deterioro cognitivo derivado de los resultados de estas dos pruebas, quisimos explorar en mayor profundidad qué dominios cognitivos están afectados en estos pacientes. Por lo tanto, se elaboró otra batería neuropsicológica más exhaustiva con la que pudimos investigar los déficit prefrontales y mnésicos. Esta batería neuropsicológica se realizó mediante diferentes pruebas que han demostrado ser las más adecuadas para la detección del deterioro cognitivo en los pacientes con TUS (Sachdeva et al., 2016). Esta estuvo compuesta por el *Montreal Cognitive Assessment* (MoCA), Test de Aprendizaje Verbal España-Complutense (TAVEC), Figura compleja de Rey Osterrieth (FCRO), Dígitos Directos e Inversos del WAIS-IV (DD, DI) y el *Trail Making Test B* (TMT B) (2ª batería neuropsicológica, Tabla). Exceptuando para el MoCA, que distingue entre DCL, moderado y severo, definimos un valor de -1 puntuación estándar (Z) como el punto de corte para el DCL y un valor de -2 puntuación estándar como el punto de corte para el deterioro cognitivo severo, como informaron estudios previos (Achiron et al., 2013; Madureira et al., 2001). Además, las puntuaciones Z se calcularon teniendo en cuenta la edad y el nivel educativo cuando las pruebas requerían.

1ª Batería neuropsicológica:

Frontal Assessment Battery

Para la evaluación de la función ejecutiva, se empleó el FAB, que ha demostrado ser una herramienta válida y fiable para el cribado de deterioro de la función ejecutiva. Además

de medir función ejecutiva, también evalúa praxias. La prueba consta de seis ítems, puntuables de 0 a 3, siendo 0 la menor puntuación y 3 la máxima. Con lo cual, la mejor calificación a la que puede optar cualquier persona que se someta a la prueba es de 18 puntos. El punto de corte para el déficit frontosubcortical se ubicó en 16-15 y el punto de corte para la demencia frontosubcortical en 13-12. A la hora de realizar los grupos estadísticos, un paciente con una puntuación de 15 se incluirá en el grupo de déficit frontosubcortical mientras que un paciente con un baremo de 12 formará parte del grupo de demencia frontosubcortical (Rodriguez del Alamo, A., Catalan Alonso, M. J. & Carrasco Marin, 2003).

Memory Failure Everyday 30

El MFE-30 test es un autotest de treinta afirmaciones diseñado para arrojar información sobre el estado memorístico de una persona. El paciente, en este caso ayudado por un evaluador cualificado, ha de calificar de 0 a 5 cuan identificado se siente con cada una de las afirmaciones, siendo 0 el mínimo y 5 el máximo. Dado que todas las afirmaciones son sobre olvidos cotidianos, cuanto más identificado se sienta el paciente con las afirmaciones, mayor será la puntuación y mayor será su deterioro. Los baremos aportados por diferentes estudios muestran que el MFE-30 es un instrumento útil en la práctica clínica apuntando que existe una estrecha relación entre la aparición de quejas cognitivas, la presencia de sintomatología prefrontal y el estrés percibido (Lozoya-Delgado, P., Ruiz-Sánchez de León, J. M., & Pedrero-Pérez, 2012). Para la interpretación clínica de los resultados se siguió el "baremo para la interpretación clínica" incluido en el anexo.

2ª Batería neuropsicológica:

Montreal Cognitive Assessment

El MoCA es una prueba breve de screening que evalúa estado cognitivo general. Fue desarrollado por Nasreddine en 2005 (Nasreddine et al., 2005) y posteriormente adaptado al español (Ojeda, 2016). Se ha vuelto una herramienta muy popular tanto en

clínica como en investigación y se utiliza como una alternativa al tradicional MMSE. Además, se ha comprobado que el MoCa presenta una mayor sensibilidad que el MMSE (Dong et al., 2010; Larner, 2012).

Evalúa múltiples dominios cognitivos que incluyen atención, concentración, funciones ejecutivas, memoria, lenguaje, habilidades visoespaciales, abstracción, cálculo y orientación, a través de siete subtests: 1) Capacidad visuoespacial/ejecutiva: se evalúa mediante una tarea de alternancia gráfica adaptada del TMT B (1 punto), con la copia de un cubo geométrico (1 punto) y con la copia del test del reloj (3 puntos); 2) Denominación: se evalúa mediante tres ítems de nominación por confrontación visual de tres animales de bajo grado de familiaridad (3 puntos), 3) Atención: consta de tres tareas: dígitos directos e inveros (2 puntos), atención sostenida (1 punto) y una serie de sustracciones (3 puntos), 4) Lenguaje: consta de dos tareas: repetición de dos frases complejas (2 puntos) y fluidez fonética, cantidad de letras que comiencen con la letra P durante un minuto (1 punto), 5) Abstracción: se compone de dos ítems de razonamiento verbal abstracto (2 puntos), 6) Aprendizaje y recuerdo diferido: consta de dos ensayos de aprendizaje de cinco palabras (no puntúan) por las que se pregunta de forma diferida a los cinco minutos (5 puntos), 7) Orientación: se evalúa la orientación temporal y espacial (6 puntos).

La puntuación total del MoCa es de 30 puntos, y el tiempo de administración es de 7-10 minutos aproximadamente, según el estado del paciente. La interpretación de la puntuación total directa depende de la edad y del nivel educativo del participante previamente definido. De este modo se obtienen los puntos de corte para considerar DCL, moderado o grave (Ojeda, N., 2016). Cuenta con una adecuada consistencia interna, sus resultados son estables en el tiempo y presenta buena fiabilidad test-retest e inter-examinadores (Gallego et al., 2009).

Test de Aprendizaje Verbal España-Complutense

El TAVEC (Benedet, M. J., & Alejandre, 1988) es una prueba utilizada para evaluación de la memoria episódica verbal. Su estructura está basada en los modelos de memoria denominados multialmacén (Atkinson, R. C., & Shiffrin, 1968). Por lo tanto, puede se puede extraer información sobre la codificación o aprendizaje, el almacenamiento a corto y largo plazo, el recuerdo/reconocimiento, y el sistema atencional ejecutivo central. El TAVEC proporciona información sobre: 1) la curva de aprendizaje, 2) los efectos de primacía y recencia, 3) la estabilidad del aprendizaje, 3) el uso de estrategias semánticas y seriales, 4) la susceptibilidad a la interferencia, 5) la retención de información a corto y largo plazo, 6) el beneficio de las claves semánticas en el recuerdo, 7) la presencia de perseveraciones e intrusiones, 8) la discriminabilidad, 9) el sesgo de respuesta.

La prueba comienza con la presentación por parte del evaluador de una lista de 16 palabras (lista A), formada por palabras pertenecientes a cuatro categorías semánticas, y la petición de su recuerdo. Este procedimiento se repite en cinco ensayos. Al finalizar el quinto ensayo, se administra una segunda lista de 16 palabras (lista B). Posteriormente, se pide el recuerdo de la lista A. Inmediatamente después, se indica una de las categorías semánticas (por ejemplo, especias) y se le pide el recuerdo de todas las palabras de la lista A que pertenezcan a esa categoría. Se hace lo mismo con las otras tres categorías semánticas. Pasados unos 20 minutos, se pide nuevamente el recuerdo libre de la lista de palabras A y, a continuación, el recuerdo por categorías semánticas. Finalmente, se administra una prueba de reconocimiento de los elementos de la lista A. Esta prueba permite obtener gran cantidad de información, no solo de los déficits en consolidación mnésica sino también de las estrategias ejecutivas implicadas en la memoria, comparando el sujeto con una muestra similar en edad (Benedet M. J., et al 1998). Además, se ha utilizado en artículos publicados en revistas de gran impacto y se ha comprobado que es más sensible que la escala de Wechler-III (WMS-III), con el que tiene una alta validez de constructo (Luna-Lario, P., Peña, J., & Ojeda, 2017).

Dígitos Directos e Inversos del WAIS-IV

Los DD y los DI del WAIS-IV evalúa la memoria de trabajo verbal, la manipulación mental, la flexibilidad cognitiva y la capacidad de memoria (span). El WAIS-IV es una prueba que ha sido ampliamente utilizada para evaluar cognición (Muñiz y Fernández-Hermida, 2010). Es capaz de distinguir entre tres grupos clínicos diferentes (Alzheimer, DCL y sujetos con quejas clínicas subjetivas), lo cual quiere decir que es sensible ante las progresivas disminuciones de la memoria de trabajo en esta población (Ruchinskas, 2019). Esta prueba consiste en dos tareas en las que se presenta una secuencia de 9 dígitos como máximo y el sujeto debe repetir los dígitos en el mismo orden (hacia delante) y orden inverso (hacia atrás). La duración de la prueba ronda los 10 minutos.

Figura Compleja de Rey Osterrieth

El test FCRO fue diseñado inicialmente por André Rey con el objetivo de evaluar la organización perceptual y la memoria visual en pacientes con lesiones cerebrales. Además, se ha usado para valorar otro tipo de patologías y actualmente es una herramienta muy usada en la evaluación del Trastorno por Déficit de Atención e Hiperactividad (Rey, 1997). La prueba consiste en copiar y después reproducir un dibujo geométrico complejo y está dirigida a sujetos con sospecha de déficits en memoria. El sujeto debe copiar el modelo de la Figura de Rey, indicándole que la reproducción no necesariamente debe ser exacta, pero que debe tener en cuenta los detalles y las proporciones. Se proporciona al sujeto una hoja y un lápiz y comienza su copia. Anotando el orden de reproducción de las distintas partes de la que consta la figura se puede descubrir, al analizar el dibujo, la marcha seguida en el proceso de copia. Si en el curso de la copia el sujeto cambia la posición del modelo, hay que volverlo a poner en la posición inicial. Finalmente, se le pregunta si ha terminado y se anota el tiempo empleado. Transcurrido un cierto tiempo de la fase de copia (un intervalo que no supere los 3 minutos) se le pide que reproduzca la figura sin tenerla a la vista, para ello se le

proporciona un folio en blanco y se controla con un cronómetro el tiempo de ejecución de la prueba.

Trail Making Test B

El TMT o test del trazo en castellano es una prueba que tiene como objetivo valorar diferentes funciones cognitivas, entre ellas atención selectiva, memoria de trabajo, impulsividad, enfoque y ejecución motora, velocidad de procesamientos psicomotor y flexibilidad cognitiva. En concreto, la parte B (TMT B) del test evalúa funciones ejecutivas y atención dividida y alternada. La tarea consiste en unir mediante líneas de forma consecutiva alternando números y letras que deben estar ordenados de forma alfabética y ascendente. Se realiza siempre una prueba previamente a los ensayos y se le pide al sujeto que no levante el lapicero del papel y que intente ir lo más deprisa posible ya que el tiempo de ejecución de la prueba se controla con un cronómetro (Rasmusson et al., 1998).

Tabla 7. Evaluación clínica y neuropsicológica de los estudios de la presente tesis.

Evaluación clínica		Exploración	Tiempo	Aplicación
Psicopatológica	*PRISM*	Datos sociodemográficos. Evaluación TUS y otros trastornos del Eje I y II del DSM-IV-TR	2-3 h	Estudios 1, 2, 3 y 4
1ª Batería neuropsicológica	*FAB*	Screening funciones frontales (función ejecutiva y praxias).	15 min	Estudios 1 y 3
	MFE	Fallos de memoria en la vida cotidiana.	10 min	
2ª Batería neuropsicológica	*MoCA*	Screening completo de deterioro cognitivo.	10 min	Estudio 4
	TAVEC	Memoria episódica verbal.	40 min	
	FCRO	Memoria episódica visoespacial.	10 min	
	TMT B	Funciones ejecutivas (flexibilidad cognitiva y atención ejecutiva).	5 min	
	DD/DI	Memoria a corto plazo y memoria de trabajo.	10 min	

3.3.3. Diseño de la evaluación:

Durante el proceso de reclutamiento, la evaluación clínica comenzaba con la entrevista psiquiátrica PRISM y terminaba con la valoración neuropsicológica. Con el objetivo de minimizar el cansancio del participante y para que no interfieran unas pruebas con otras, la evaluación podía dividirse en dos sesiones (evaluación psicológica/ neuropsicológica) dependiendo de la complejidad del paciente o su disponibilidad. Se estableció un orden en el que se presentaron las pruebas para que no se produjeran interferencias entre ellas, y para reducir la variabilidad de las evaluaciones entre los distintos evaluadores. Los tiempos que se exponen a continuación son aproximados, ya que depende de la disposición, el cansancio y las capacidades cognitivas de cada individuo.

En primer lugar, durante las primeras 2-3 h se recogieron los datos sociodemográficos, se evaluó la presencia o ausencia de uno o varios TUS y la comorbilidad psiquiátrica asociada mediante la entrevista PRISM. En segundo lugar, se aplicó la 1ª batería neuropsicológica en las primeras etapas del reclutamiento, en la cual se administró primero el FAB (15 min) y después el MFE (10 min). En etapas finales del reclutamiento, se procedió a la aplicación de la 2º batería neuropsicológica, administrándose la prueba de screening MoCA (10 min) como inicio de la evaluación neuropsicológica. Después de esta prueba, se aplicaba la primera parte del TAVEC, que incluye aprendizaje, recuerdo inmediato libre y con claves de información verbal (10 min). Durante los 25 minutos de la pausa del TAVEC, se administraba la FCRO, el TMT B y DD/DI, ya que son tareas visoespaciales o de contenido numérico que no interfieran con la memoria episódica verbal. Dentro de los 3 min de pausa de la Figura Compleja de Rey se administró el TMT B y después de finalizar la memoria de la FCR, se realizaron los DD/DI del WAIS-IV. Acto seguido, se finaliza la segunda parte del TAVEC, que incluye recuerdo diferido libre y con claves, y reconocimiento (10 min) (Figura 18).

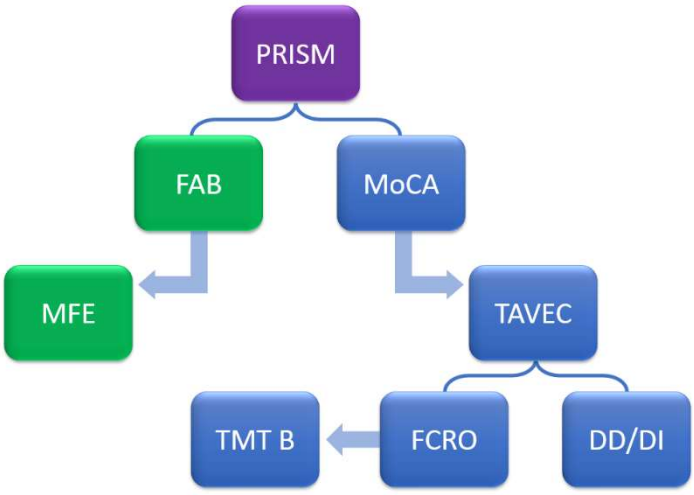


Figura 18. Diseño de la evaluación clínica y neuropsicológica de los estudios de la presente tesis. En morado consta la evaluación clínica, en verde la 1ª batería psicológica y en azul la 2ª batería neuropsicológica.

3.4. Obtención y procesamiento de la sangre

Las muestras de sangre de cada paciente se recogieron por la mañana a primera hora (de 8:30 a 11:00h) por el equipo de enfermería, tras un periodo de ayuno de 8 a 12 horas y previa prueba de alcoholímetro para descartar consumo reciente de alcohol, el día de la entrevista psiquiátrica u otro diferente, según los horarios de los pacientes. A partir de estas muestras se determinaron las concentraciones de diferentes moléculas circulantes. La sangre periférica fue extraída y transportada en frío en tres tubos de 10 mL con K2 EDTA (BD, Franklin Lakes, NJ, EE. UU.). Las muestras de sangre fueron inmediatamente procesadas para la obtención del plasma a través de su centrifugación a 2200 xg durante 15 minutos (4°C).

Las muestras plasmáticas válidas fueron fraccionadas en varios criotubos perfectamente codificados y se almacenaron a -80°C hasta su posterior análisis. Uno de los tubos de sangre individual de cada paciente perteneciente al grupo alcohol se enviaba el día de la extracción al Biobanco de la Red de Trastornos Adictivos para futuros estudios genómicos. Junto a la muestra se enviaba un registro de datos sociodemográficos, tiempo de abstinencia, la exploración de trastornos mentales y de sustancias necesarias para rellenar el

registro en su base de datos. Este envío se realizaba de manera urgente desde el hospital y lo recibían en Alicante a primera hora de la mañana para su correcto almacenamiento. Estas muestras están siendo analizadas en la actualidad para identificar polimorfismos genéticos en los genes candidatos responsables de la producción y función de los biomarcadores seleccionados, y constituyen una de las investigaciones derivadas de esta Tesis Doctoral.

3.5. Determinación de parámetros bioquímicos

Cuantificación de factores neurotróficos circulantes:

Se midieron las concentraciones de las moléculas de estudio BDNF y NT-3 mediante el uso de Kits comerciales de) enzimoinmunoanálisis (ELISA) específicos para cada molécula en humanos. Teniendo en cuenta que todos los protocolos eran similares, difiriendo tan solo en particularidades procedimentales de cada casa comercial, pero manteniendo idéntico fundamento bioquímico. A continuación, se explica el protocolo que se siguió para medir las concentraciones de BDNF en el plasma humano sirviendo, así como modelo explicativo del método.

Para realizar el ensayo, se añaden a tantos pocillos como sean necesarios (hasta un máximo de 96) las muestras y el estándar preparado (necesario para realizar la curva patrón por el método de diluciones sucesivas), seguido de la solución con el anticuerpo. Tras una primera incubación, se emplea el tampón de lavado para lavar los pocillos mediante aspiración o decantación, y así eliminar el material indeseado presente en la muestra. Se añade el sustrato TMB (3, 3', 5, 5'-tetrametil-benzidina) y durante la incubación se producirá una reacción de coloración catalizada por la peroxidasa HRP, (Horse Radish Peroxidase) que en este protocolo va incluida en la solución de sustrato TMB. Tras un período de 10 minutos en el que se produce la reacción de coloración, se paraliza dicha reacción con la adición de la solución de parada, tornando cada pocillo a una coloración amarilla. La señal generada es proporcional a la cantidad del analito a medir, en nuestro caso BDNF. Las placas se leyeron a una longitud de 450nm en un espectrofotómetro

Hitachi 737 Automatic Analyzer (Hitachi Ltd., Tokyo, Japan). El protocolo descrito como ejemplo y representado en la Figura 19 corresponde a la referencia: (#Ab212166 Human BDNF SimpleStep ELISA Kit). El resto de los kits se encuentran detallados en los artículos adjuntos.

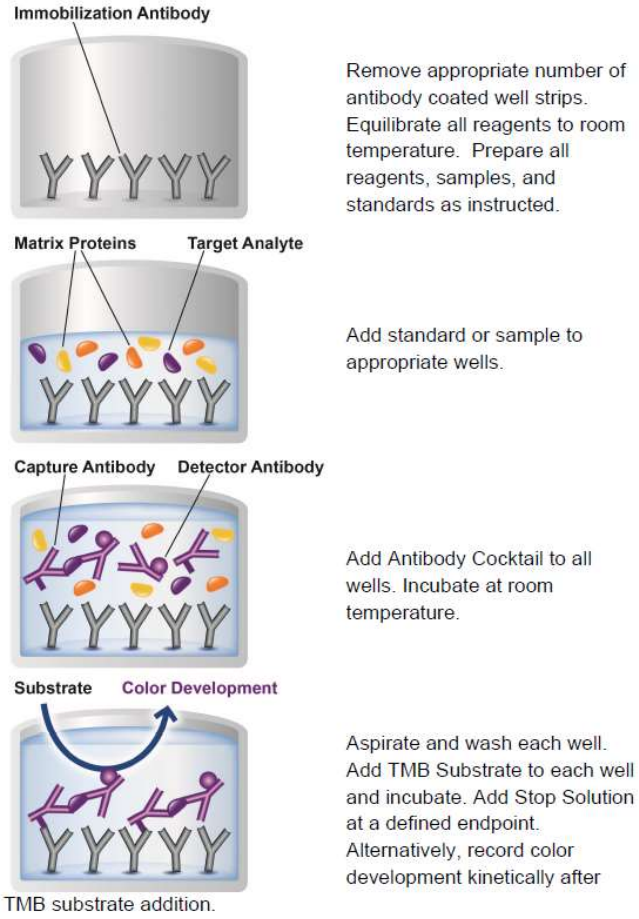


Figura 19. Representación del protocolo general de un ensayo por ELISA. Figura extraída del protocolo del Kit de BDNF con referencia #Ab212166 Human BDNF SimpleStep ELISA Kit.

Cuantificación del factor de VEGFA y quimioquinas circulantes:

La cuantificación de VEGFA y de las quimioquinas SDF-1, eotaxina, MIP-1, MCP-1, fractalquina se realizó mediante inmunoensayo simultáneo (tecnología de multiplexado). Los inmunoensayos multiplex de Invitrogen ProcartaPlex utilizan la tecnología Luminex xMAP (perfil multianalito) para permitir la detección y cuantificación simultáneas de hasta 65 objetivos proteicos en una única muestra de plasma, suero, sobrenadantes de cultivos celulares y otros fluidos corporales de 25 a 50 µL. La tecnología Luminex utiliza esferas de

captura diferencialmente para cada objetivo en un ensayo tipo ELISA multiplex. Los inmunoensayos ProcartaPlex permiten el análisis simultáneo de múltiples proteínas en una sola muestra de una amplia gama de fuentes biológicas. Combinan las eficiencias de la multiplexación con la precisión, sensibilidad, reproducibilidad y simplicidad que el ELISA.

Los kits de ensayo multiplex ProcartaPlex se realizan de la misma manera que los ELISA (Figura 20). Los pasos son similares, con la excepción de que se utilizan esferas recubiertas de anticuerpo para capturar el analito, en lugar de tener anticuerpos de captura unidos directamente a la placa. Cada kit proporciona estándares de concentración conocida para que se pueda establecer una curva estándar. Después de la incubación en un agitador, las perlas se lavan colocando la placa de 96 pocillos en un imán plano durante 30 segundos, después de lo cual el líquido se desecha agitando los pocillos o utilizando un lavador de placas automático. Se retira el imán y las esferas se resuspenden en el anticuerpo de detección. Después de otra incubación y lavado, le sigue la adición de estreptavidina-R-ficoeritrina (SAPE). A continuación, las esferas se lavan y están listas para analizar.

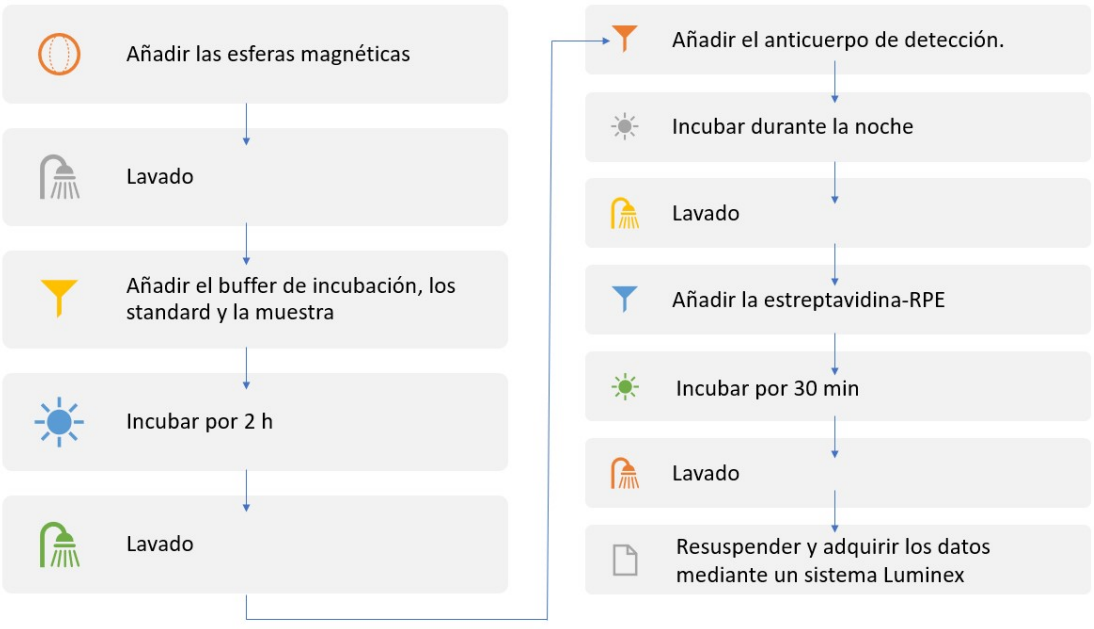


Figura 20. Representación de un protocolo general de un ensayo por multiplexado.

Como hemos dicho, nuestro objetivo fue analizar las concentraciones plasmáticas de SDF-1, eotaxina, MIP-1, MCP-1, fractalquina y VEGFA utilizando un kit de inmunoensayo

de esferas 7-ProcartaPlex personalizado para humanos (Invitrogen, cat. No. PPX-07-MXH6ANW). Las placas se leyeron en el Luminex xMAP ® tecnología - sistema MAGPIG (ThermoFisher). La sensibilidad fue de aproximadamente 13, 33, 12, 51 y 39 pg / ml para eotaxina, MIP-1α, MCP-1, fractalquina y VEGFA respectivamente. La variación media intraensayo (% de réplicas de CV) fue de 5,3, 9,3, 10,3, 7,1 y 11,1 respectivamente, y la variación media entre ensayos (% CV) fue 34,4, 80,1, 59,2, 106,03 y 36,7 respectivamente para todos los análisis. Los valores de concentración mínima detectable se atribuyeron a los valores perdidos que estaban por debajo de la curva estándar.

Cuantificación de neurofilamentos

Los niveles de NfLs han sido recientemente implicados en la prognosis de COVID-19 severo y han sido determinados utilizando un inmunoensayo digital y el SIMOA HD1 Analyser platform (Prudencio M, Erben Y, Marquez CP, Jansen-West KR, Franco-Mesa C et al., 2021; Serrano-castro et al., 1867), por lo que se han evaluado siguiendo la misma metodología en la presente Tesis Doctoral.

3.6. Análisis estadísticos

Todos los datos de las tablas y gráficos fueron expresados en número y porcentajes de sujetos [n (%)], medias y desviación estándar de las concentraciones [media (SD)] o valores individuales de variables (correlaciones). Los análisis estadísticos se realizaron mediante los programas SPSS versión 22 (IBM, Amonk, NY, EE.UU.) y *Graph-PadPrism* versión 8 (GraphPad Software, San Diego, CA, EE.UU.). Consideramos un valor $p<0,05$ como estadísticamente significativo.

Análisis de correlaciones

Para los análisis de correlación lineal de dos variables aleatorias cuantitativas se empleó el "coeficiente de correlación de Pearson" (r) en supuestos de normalidad y se empleó el "coeficiente de Spearman" (rho) para variables aleatorias continuas que no cumplen los supuestos de normalidad.

Análisis y distribución de medias

Para las variables cualitativas nominales y ordinales se utilizaron diferentes pruebas de *chi-cuadrado* según el número de categorías y el tamaño de la muestra: a) Chi-cuadrado cuando había más de dos categorías y un tamaño muestral alto para cada grupo [N> 5 (%)], b) Chi-cuadrado por tendencia o lineal por asociación lineal cuando había más de dos categorías y tamaño muestral pequeño para cada grupo [N <5 (%)], c) Prueba exacta de Fisher para dos categorías y un tamaño muestral pequeño.

Para variables cuantitativas se empleó la prueba t de Student para análisis de diferencias significativas entre dos grupos y la prueba de Análisis de la Varianza ANOVA con la prueba p*ost-hoc* de Tukey para variables divididas en más de dos grupos. Para comprobar el supuesto de normalidad utilizamos Shapiro Wilk para un tamaño muestral <50 y Kolmogorov Smirnov para un tamaño muestral >50. En aquellas muestras que no cumpliesen los criterios de normalidad, en lugar de aplicar la prueba t de Student se aplicó la U de Mann-Whitney o Kruskal Wallis dependiendo de la comparación entre dos o más grupos.

Análisis de la covarianza

Se empleó el análisis de la covarianza (ANCOVA), modelo lineal general con una variable cuantitativa y uno o más factores. Mediante este análisis se consigue la homogeneidad en la variable dependiente debido a la influencia de las variables cuantitativas que se incluyan en el modelo. Un aumento de covariables puede elevar la potencia estadística. Para respetar la normalidad de las distribuciones, se realizaron transformaciones logarítmicas de los datos. Como análisis *post-hoc* se realizó la prueba de Bonferroni.

Análisis de regresión logística binaria

Con la finalidad de determinar variables que pudieran discriminar entre dos grupos poblacionales, se realizó un análisis de regresión logística binaria mediante la prueba Chi-

cuadrado de Pearson ($\chi 2$), cumpliendo con la prueba de Hosmer-Lemeshow. Para comprobar el supuesto de multicolinealidad examinamos la Tolerancia y el Factor de Inflación de Varianza (VIF). El valor de corte para la Tolerancia fue >0,10 y <10 para VIF. El poder discriminativo del modelo logístico y los umbrales de determinadas variables fueron evaluados por mediante el análisis de las características operativas del receptor (ROC) considerando el área bajo la curva (AUC).

Análisis de componentes principales

Para determinar los diferentes perfiles de pacientes que acudieron a tratamiento ambulatorio se realizó un análisis de componentes principales con rotación varimax y relaciones bivariadas (correlación). Solo variables con carga factorial de al menos 0,3 (compartiendo al menos el 10% de la varianza con un factor) se utilizaron para la interpretación.

4. Resultados

A continuación, se presentan en el orden señalado los estudios publicados o enviados a publicación que describen y discuten parcialmente los objetivos de la presente tesis doctoral. Al final de cada artículo se listan una serie de conclusiones parciales.

4.1. Estudio 1:

Evaluación de factores neurotróficos y nivel educativo como predictores de deterioro cognitivo en el trastorno por consumo de alcohol.

“Evaluation of neurotrophic factors and education level as predictors of cognitive decline in alcohol use disorder”.

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Evaluation of neurotrophic factors and education level as predictors of cognitive decline in alcohol use disorder

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Cognitive reserve (CR) is the capability of an individual to cope with a brain pathology through compensatory mechanisms developed through cognitive stimulation by mental and physical activity. Recently, it has been suggested that CR has a protective role against the initiation of substance use, substance consumption patterns and cognitive decline and can improve responses to treatment. However, CR has never been linked to cognitive function and neurotrophic factors in the context of alcohol consumption. The present cross-sectional study aims to evaluate the association between CR (evaluated by educational level), cognitive impairment (assessed using a frontal and memory loss assessment battery) and circulating levels of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) in patients with alcohol use disorder (AUD). Our results indicated that lower educational levels were accompanied by earlier onset of alcohol consumption and earlier development of alcohol dependence, as well as impaired frontal cognitive function. They also suggest that CR, NT-3 and BDNF may act as compensatory mechanisms for cognitive decline in the early stages of AUD, but not in later phases. These parameters allow the identification of patients with AUD who are at risk of cognitive deterioration and the implementation of personalized interventions to preserve cognitive function.

Cognitive decline is currently considered a global health problem. The World Health Organization (WHO) recognizes dementia as a public health priority, positioning it as one of the main diseases of the twenty-first century. The prevalence of dementia is predicted to markedly increase in the next 30 years, affecting 131.5 million people by 2050[1]. Therefore, the WHO has endorsed a *global action plan on the public health response to dementia 2017–2025* for the development of a coordinated global response to effectively address the issue of this disease[2].

Although high alcohol consumption has been associated with an acceleration of cognitive decline in aging[3], the WHO considers alcohol consumption to be one of the many risk factors for dementia and not a main component in the etiology of the disease. However, Schwarzinger et al.[4] found that alcohol use disorder (AUD) is the most robust risk factor for the onset of any type of dementia, especially early onset dementia. More strikingly, in a recent epidemiological study, the most common diagnosis in cases of early-onset dementia was alcohol-related dementia (18.4%), followed by Alzheimer's disease (17.7%), vascular dementia (12.8%) and frontotemporal dementia (11.3%)[5]. In addition, differences in alcohol consumption habits have been shown to affect the risk of dementia in the absence of genetic factors explaining these associations[6].

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Regarding preventive mechanisms, the concept of cognitive reserve has been developed to explain the discrepancy between the extent of brain pathology and the expected cognitive performance of an individual[7]. Cognitive reserve is understood as an active mechanism to address brain damage that depends on the creation of flexible and effective neural networks as a result of cognitive stimulation throughout life[8]. Thus, cognitive reserve seems to be the result of innate intelligence, years of schooling, occupation, life experiences, social relationships and leisure activities[9–11]. However, one of the most commonly used measures of cognitive reserve is years of education because it allows the other factors to be inferred[12]. For example, it has been shown that a high education level softens the clinical manifestations and the progression of mild cognitive impairment[13]; in addition, it has been related to fewer alterations in the biomarkers t-tau, p-tau, t-tau/AB42, and p-tau/AB42 in individuals with Alzheimer's disease[14].

Thus, there is evidence that a greater reserve can act as a protective factor against normal aging processes[15]; the appearance of neuropathological diseases, such as mild cognitive impairment[16], Alzheimer's disease[17,18], Parkinson's disease[19], multiple sclerosis[20], brain damage related to trauma, stroke or tumor[9,21]; and psychiatric disorders, such as depression, bipolar disorder, anxiety and psychosis[22,23]. In addition, the concept of cognitive reserve has recently been established in the field of addictions, and research suggests that it has a preventive role against the onset of drug use and is associated with less severe consumption patterns, less cognitive decline and better response to treatment[24].

Along these lines, it is widely known that chronic alcohol consumption can affect the central nervous system (CNS) and cause the deterioration of cognitive functions[25]. This deterioration has a neurodegenerative component that is due not only to the nutritional deficiencies (i.e., thiamine deficiency) of chronic drinkers but also to neurotoxicity mediated by inflammatory phenomena that are directly activated by alcohol, including the activation of TL4 receptors, the release of proinflammatory cytokines and the onset of oxidative stress[26,27]. These phenomena involve the activation of the brain's resident immune system through microglial cells and astrocytes, which leads to the emergence of neuronal apoptosis and even necrosis in the central nervous system[28,29]. Postmortem studies have revealed a significant loss of gray and white matter in the brains of people with AUD, especially in the prefrontal cortex and cerebellum[30].

Regarding cognitive integrity, patients who have chronic alcohol consumption show abnormalities in motor control, processing speed, sustained attention, memory and learning and overall executive function[3,31,32], with visuospatial cognition being the most affected component[33,34]. Specifically, patients with AUD show deficits in tasks that require cognitive control, cognitive flexibility, inhibition, planning and working memory[35,36]. Additionally, these patients exhibit changes in emotional processing and social cognition[37]. It is important to mention that AUD is often accompanied by other substance use disorders and psychiatric disorders that, when added to cognitive deficits, severely worsen the patient's clinical situation, leading to even greater social stigmatization, withdrawal from health services and, ultimately, social exclusion[25,38,39].

On the other hand, growth factors are a set of proteins that play a crucial role in cell growth, proliferation and differentiation in the CNS and are important to cognitive processes[40]. Among the neurotrophic factors are neurotrophins, including brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4. The functions attributed to BDNF include the regulation of neurogenesis, synaptogenesis and gliogenesis and the control of long-term potentiation (LTP) mechanisms in the hippocampus that result in increases in memory and cognition[41]. Clinical studies have described the decrease in BDNF levels and cognitive decline in patients with psychiatric disorders such as depression, schizophrenia and bipolar disorder[42] and in patients with neuropathological diseases such as epilepsy[43], mild cognitive impairment, Alzheimer's disease[44] and Parkinson's disease[45]. In addition, our group has recently associated BDNF with the cognitive deterioration of patients with AUD during abstinence[46]. NT-3 has been associated with proliferation, migration and neuronal differentiation, although it does not appear to be involved in CNS maturation, and little is known about its role in cognition[47]. In a preclinical model, deregulated NT-3 expression was found in both the neonatal brain and the adult hippocampus, which could explain the cognitive deficits of Down syndrome[48]. In a clinical study, the rs6332 polymorphism of NT-3 was associated with executive function in patients with Alzheimer's disease[49]. Additionally, insulin-like growth factors (IGF1 and IGF2) are another family of neurotrophic proteins involved in the regulation of the proliferation, development and growth of neuronal cells, whose circulation is decreased in patients with AUD[50].

Given that the longevity of the human population is increasing and that the data on alcohol consumption continue to be alarmingly high, in the present study, we wanted to identify the impact of cognitive reserve (evaluated by educational level) and the circulating neurotrophic factors as decisive elements in the onset and progression of cognitive decline in patients diagnosed with AUD who attend outpatient treatment. To this end, we conducted a cross-sectional study to evaluate the associations between education level (primary, secondary and university), the presence of cognitive decline and circulating levels of neurotrophic factors BDNF, NT-3, IGF-1, IGF-2 and its associated protein IGFBP-3 in alcohol-abstinent patients with AUD.

Results

Sociodemographic characteristics of the sample. Table 1 presents the sociodemographic description of the total sample of alcohol-abstinent patients and the subcohort with a complete neuropsychological battery (FAB and MMSE), all of whom were selected from outpatient programs for AUD. Of the total study sample, 74.4% were men, and 25.4% were women; their mean age was 49.23 years (SD = 8.87), and their body mass index was 25.99 kg/m^2 (SD = 4.36). A total of 28.2% of the population was single, 37.8% was married or had a partner, and 30.9% was divorced or separated. Regarding educational level, 37% had a primary education, 42.2% had a secondary education, and 16.8% had a university education. A total of 24% were employed, compared to 45.4% who were unemployed, and 30.5% were on sick leave, were retired or were homemakers. As Table 1 shows, the

Variables	Patients with alcohol use disorder				
	Total sample N = 262	Subcohort[a] N = 58	Statistics	df	p-value
Age (mean ± SD) (years)	49.23 ± 8.87	49.57 ± 8.20	− 0.270[b]	318	0.787
Body mass index [mean (SD)] (kg/m^2)	25.99 ± 4.36	25.86 ± 3.81	0.210[b]	318	0.819
Sex [N (%)]					
Women	67(25.4)	11 (19)	1.125[c]	1	0.316
Men	195 (74.4)	47 (81)			
Marital status [N (%)]					
Single	74 (28.2)	14 (24.1)	2.696[c]	4	0.610
Cohabiting	99 (37.8)	20 (34.5)			
Separated	81 (30.9)	22 (37.9)			
Widow	8 (3.1)	2 (3.4)			
Education level [N (%)]					
Elementary	97 (37)	22 (37.9)	0.240[c]	2	0.887
Secondary	121 (46.2)	25 (43.1)			
University	44 (16.8)	11 (19)			
Occupation [N (%)]					
Employed	63 (24)	11 (19)	2.416[c]	4	0.660
Unemployed	119 (45.4)	30 (51.7)			
Other	80 (30.5)	17 (29.3)			

Table 1. Sociodemographic characteristics of the total sample of patients. *df* degree of freedom. [a]Patients with neuropsychological battery (FAB and MMSE). [b]Chi-square test statistic. [c]Student's t test statistic.

subcohort of patients had sociodemographic characteristics that were very similar to those of the total sample, indicating that it was a representative subcohort for this group of variables.

Variables associated with alcohol consumption. The variables related to alcohol consumption in the total AUD group and the subcohort with a complete neuropsychological battery (FAB and MMSE) were evaluated and are described in Table 2. Of the total study sample, the mean age at the start of alcohol consumption was 16.26 years (SD = 3.9), and the mean age when dependence developed was 29.56 years (SD = 10.62). The mean number of alcohol addiction severity criteria (range 0–11) was 7.46 (SD = 2.19), the mean duration of abstinence from alcohol at the time of evaluation was 306.67, and the mean length of AUD diagnosis was 15.35 years (SD = 10.41). The AUD group had a high prevalence of other substance use disorders (40.5%), with cocaine use disorder being the most prevalent (29.4%). In addition, a high prevalence of other psychiatric disorders was observed (68.3%). Thus, in the AUD group, 45.4% and 39.3% were diagnosed with mood and anxiety disorders, respectively, at some point in their lives. The alcohol-abstinent patients had received the following psychotropic medications for at least 12 months: antidepressants (43.5%), anxiolytics (39.3%) and anticraving medications (27.1%). Lastly, 64.1% of the patients were treated with disulfiram. As Table 1 shows, the subcohort of patients with the complete neuropsychological battery (FAB and MMSE) has some sociodemographic characteristics that were very similar to those of the total sample, indicating that they were representative in terms of the variables related to alcohol use and psychiatric comorbidity. The patients in the subcohort differed from the total sample only in that they had a shorter duration of abstinence at the time of evaluation (128.61 days; $t_{312} = 2.443$, $p = 0.003$).

Cognitive impairment and education level in alcohol-abstinent patients. The neuropsychological evaluations of the subcohort of 58 alcohol-abstinent AUD patients showed that 75.9% had some type of cognitive impairment. Thus, 74.1% of the patients had some deficit related to frontal lobe function (evaluated with the FAB), and 36.2% of them had memory deficits (evaluated with the MFE). Specifically, 48.3% seemed to have signs of cognitive impairment similar to frontosubcortical deficit, and 25.9% had signs of impairment similar to frontosubcortical dementia, as indicated in the manual of the instrument[51]. However, 58.6% had insignificant memory problems, 19% had mild memory impairment that had little impact on daily life, and 17.2% had severe memory impairment with a significant impact on daily life, as indicated in the manual[52].

The influence of educational level on cognitive status was examined using two-way ANCOVA with "education level" as a factor and "age" as a covariate. We verified that the data met the statistical assumptions, and logarithmic transformations were performed on the variables that did not meet the assumptions. As shown in Fig. 1, there were statistically significant differences in FAB scores as a function of education level (primary, secondary, university) ($F_{2,58} = 4.850$, $p = 0.012$); patients with a primary education level had lower FAB scores than those with a university education level ($p = 0.011$). However, we did not find statistically significant differences in the MMFE score as a function of education level ($F_{2,58} = 0.608$, $p = 0.548$).

Variables	Patients with alcohol use disorder				
	Total sample N = 262	Subcohort N = 58	Statistics[a]	df	*p*-value
Age at onset of consumption [mean (SD)] (years)	16.26 (3.9)	15.17 (2.95)	1.670[b]	314	0.096
Age at the development of dependence [mean (SD)] (years)	29.56 (10.62)	31.23 (11.07)	−0.946[b]	347	0.339
Length of AUD diagnosis [mean (SD)] (years)	15.35 (10.41)	16.36 (14.93)	−0.051[b]	295	0.964
Severity criteria [mean (range)]					
Criteria (0–11)	7.46 (2.19)	7.88 (2.08)	−1.285[b]	316	0.200
Duration of abstinence [Mean (mode)] (Days)	306.67 (60)	128.61 (60)	2.443[b]	312	**0.003**
Comorbid substance use disorders [N (%)]					
Cocaine	77 (29.4)	19 (32.8)	0.326[c]	1	0.633
Cannabis	34 (13)	6 (10.3)	0.266		0.825
Sedatives	18 (6.9)	5 (8.6)	0.245		0.579
Comorbid psychiatric disorders N (%)]					
Mood	119 (45.4)	25 (43.1)	0.024[c]	1	1
Anxiety	81 (30.9)	20 (34.5)	0.441		0.529
Personality	43 (16.4)	5 (8.6)	2.071		0.221
ADHD	54 (20.6)	6 (10.3)	3.028		0.217
Psychiatric medication use [N (%)]					
Antidepressants	114 (43.5)	31(53.4)	1.821[c]	1	0.189
Anxiolytics	103 (39.3)	19 (32.8)	0.933		0.370
Anticraving	71 (27.1)	19 (32.8)	0.713		0.420
Disulfiram use [N (%)]	168 (64.1)	45 (77.6)	0.704[c]	1	0.590

Table 2. Variables associated with alcohol consumption and psychiatric comorbidity. *df* degree of freedom. Bold values are statistically significant for $p < 0.05$. [a]The statistical analysis was conducted using the logarithmic transformed values to ensure that statistical assumptions were met for age at the onset of consumption, age at the development of dependency, duration AUD, severity criteria met and duration of abstinence. [b]Student's t test statistic. [c]Chi-square test statistic.

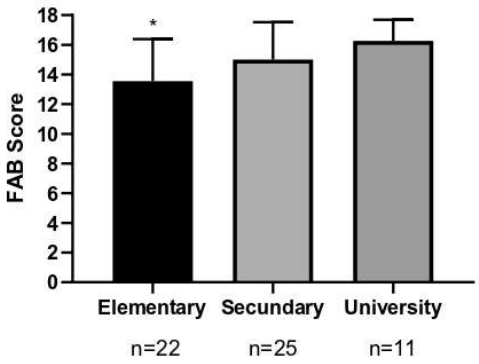

Figure 1. FAB scores according to education level (primary, secondary and university). There were statistically significant differences in FAB scoresbetweenthe primary and university levels of education assessed using a two-way ANCOVA with with "educational level" as a factor and "age" as covariate. The bars are estimated marginal means and 95% confidence intervals. *$p < 0.05$. *FAB* frontal assessment battery.

Cognitive impairment and psychiatric comorbidity in alcohol-abstinent AUD patients. To determine whether the cognitive impairment of alcohol-abstinent AUD patients was influenced by the presence of psychiatric comorbidity, we examined the effect of mood and anxiety comorbid disorders in FAB and MMSE scores using a Student's t test, since these were the most common psychiatric disorders in the study population (see Table 2). We did not find statistically significant differences in FAB or MMSE scores between patients with and without mood and anxiety disorders (see Supplementary Tables S1 and S2).

Education level and variables related to alcohol consumption. The influence of education level on the variables associated with alcohol consumption was studied using ANOVA with "education level" as a factor

Variables	Subcohort (N = 58)					
	Elementary N = 22	Secondary N = 25	University N = 11	ANCOVA (statistics)[a]		
				F-value	df	*p*-value
Age at onset of consumption [mean (SD)] (years)	14.15 (2.28)	15.24 (2.09)	17.36 (4.48)	3.175	2.56	**0.050**
Age at the development of dependency [mean (SD)] (years)	26.25 (8.51)	32.24 (12.03)	38 (9.32)	4.994	2.52	**0.010**
Length of AUD diagnosis [mean (SD)] (years)	16.15 (10.93)	19.56 (18.86)	9.45 (8.25)	0.176	2.53	0.893
Severity criteria [mean (range)]						
Criteria	8 (2.07)	8 (2.06)	7.36 [2–11]	0.364	2.57	0.697
Duration of abstinence [mean (range)] (days)	140.95 [0–1270]	149.92 [14–149.92]	56.64 [14–120]	1.424	2.56	0.250

Table 3. Variables related to alcohol consumption according to education level. *df* degree of freedom. Bold values are statistically significant for $p < 0.05$. [a]Statistical analysis was conducted on the logarithmic transformed values to ensure that statistical assumptions were met.

Variables	Subcohort (N = 57)				
	Cognitive impairment N = 43	No cognitive impairment N = 14	ANCOVA (statistics)[a]		
			F-value	df	*p*-value
Age at onset of consumption [mean (SD)] (years)	14.66 (2.64)	16.93 (3.17)	2.533	54	**0.014**
Age at the development of dependence [mean (SD)] (years)	31.37 (11.36)	30.87 (10.61)	−0.148	56	0.883
Length of AUD diagnosis [mean (SD)] (years)	18.24 (16.30)	11.20 (8.83)	−3.028	51	**0.004**
Severity criteria [mean (range)]					
Criteria	7.43 (2.03)	9.13 (1.73)	2.900	55	**0.005**
Duration of abstinence [mean (range)] (days)	157.98 [0–1440]	46.40 [14–120]	−3.184	54	**0.002**

Table 4. Education level and cognitive decline in variables associated with alcohol. *df* degree of freedom. Bold values are statistically significant for $p < 0.05$. [a]Statistical analysis was conducted on the logarithmic transformed values to ensure statistical assumptions for age at onset of consumption, length of AUD diagnosis and length of abstinence.

(Table 3). We verified that the data met the statistical assumptions, and logarithmic transformations of the variables that were not normally distributed were performed. There were significant differences in the age at onset of consumption ($F_{2,55} = 3.175$, $p = 0.050$) and the age at the development of dependence ($F_{2,55} = 4.994$, $p = 0.010$) according to education level. Thus, patients with a primary education level started consuming alcohol 3.21 years earlier ($p = 0.046$) and developed alcohol dependence 11.75 years earlier than those with a university education level ($p = 0.009$).

Cognitive impairment and variables related to alcohol consumption. To explore the effect of cognitive impairment on the variables associated with alcohol consumption, Student's t test was used (Table 4). We verified that the data met the statistical assumptions, and logarithmic transformations of the variables that did not meet the assumptions were performed. We found statistically significant differences in the age at onset of consumption ($t_{54} = 2.533$, $p = 0.014$), the length of AUD diagnosis ($t_{51} = -3.028$, $p = 0.004$), the severity criteria of addiction ($t_{55} = 2.900$, $p = 0.005$) and the duration of abstinence at the time of the psychiatric evaluation ($t_{54} = -3.184$, $p = 0.002$). Thus, patients with cognitive impairment started consuming alcohol an average of 2 years before patients without cognitive impairment, and patients with cognitive impairment had been living with the disorder 7 years longer than patients without cognitive impairment. However, patients without cognitive impairment showed greater severity of addiction [9 vs. 7 criteria (0–11)] and a shorter duration of abstinence (158 vs. 46 days) at the time of the psychiatric evaluation.

Education level and plasma concentrations of BDNF, NT-3, IGF-1, IGF-2 and IGFBP-3 in alcohol-abstinent AUD patients. The influence of education level on plasma levels of BDNF, 3-NT, IGF-1, IGF-2 and IGFBP-3 was evaluated using a two-way ANCOVA with "educational level" as a factor and "age" and "BMI" as covariates (see Supplementary Table S3). We confirmed that the data met the statistical assumptions, and logarithmic transformations were performed for values that did not meet the assumptions.

As observed in Fig. 2A, plasma concentrations of 3-NT were significantly affected by the education level ($F_{2,58} = 3.654$, $p = 0.033$). Plasma concentrations of total 3-NT were significantly lower in patients with a university education level than in patients with a primary education level ($p = 0.028$). In contrast, as shown in Fig. 2B, education level did not affect plasma concentrations of BDNF ($F_{2,58} = 0.147$, $p = 0.863$), IGF-1 ($F_{2,58} = 0.683$, $p = 0.509$), IGF-2 ($F_{2,58} = 2.662$, $p = 0.079$) or IGFBP-3 ($F_{2,58} = 1.144$, $p = 0.326$).

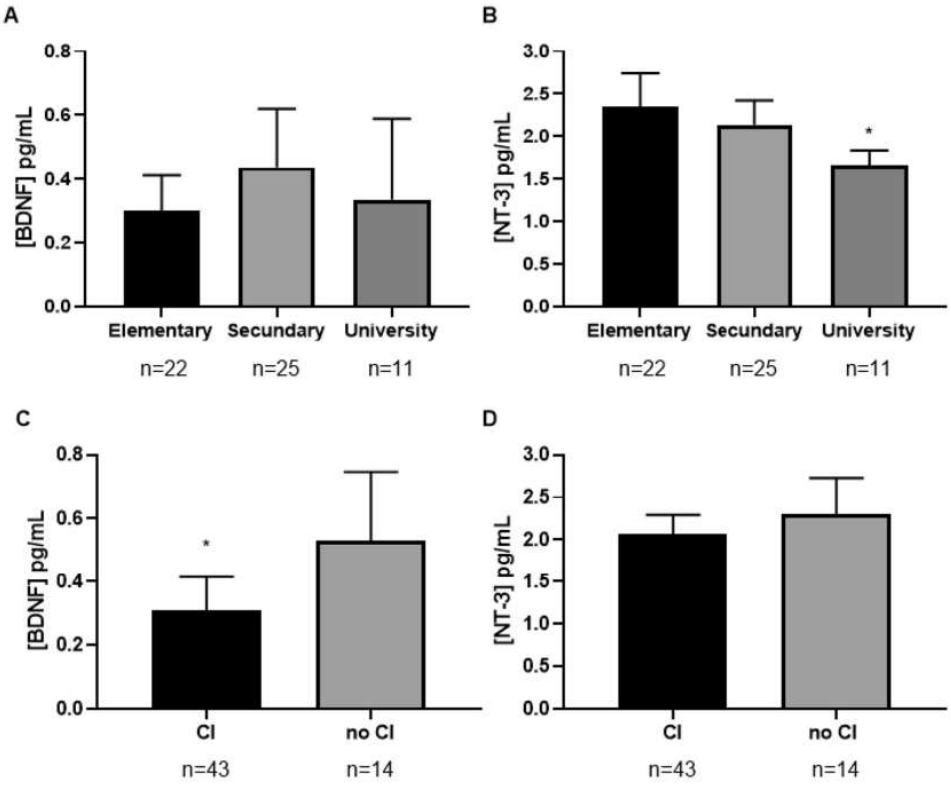

Figure 2. Plasma concentrations of either, BDNF or NT-3 in the sample according to education level and cognitive impairment evaluated using a two-way ANCOVA with "educational level" as a factor and "age" and "BMI" as covariates. Figure (**A**) shows that BDNF concentrations were not influenced by educational level. Figure (**B**) shows statistically significant differences in 3-NT concentrations between primary and university education levels. Figure (**C**) shows statistically significant differences in BDNF concentrations between patients with and without cognitive impairment. Figure (**D**) shows that NT-3 concentrations were not influenced by cognitive impairment. The bars are estimated marginal means and 95% confidence intervals. $^{*}p<0.05$. *CI* cognitive impairment.

Cognitive impairment and plasma concentrations of BDNF, NT-3, IGF-1, IGF-2 and IGFBP-3 in alcohol-abstinent AUD patients. The effect of cognitive impairment on plasma levels of BDNF, 3-NT, IGF-1, IGF-2 and IGFBP-3 was evaluated using Student's t test (see Supplementary Table S4). We confirmed that the data met the statistical assumptions, and logarithmic transformations were performed for values that did not meet the assumptions.

As Fig. 2C shows, plasma BDNF concentrations were significantly affected by the presence of cognitive impairment ($t_{56}=2.62$, $p=0.011$). Thus, plasma concentrations of total BDNF were significantly lower in patients with cognitive impairment than in patients without cognitive impairment. However, as Fig. 2D shows, cognitive impairment did not significantly affect plasma concentrations of 3-NT ($t_{56}=1.28$, $p=0.206$), IGF-1 ($t_{56}=0.294$, $p=0.770$), IGF-2 ($t_{56}=0.070$, $p=0.944$) or IGFBP-3 ($t_{53}=0.515$, $p=0.609$).

Psychiatric comorbidity and plasma concentrations of BDNF, NT-3, IGF-1, IGF-2 and IGFBP-3 in alcohol-abstinent AUD patients. To determine whether the plasma concentrations of BDNF, NT-3, IGF-1, IGF-2 and IGFBP-3 in alcohol-abstinent AUD patients were influenced by the presence of psychiatric comorbidities, we used Student's t test to examine the differences between patients with and without mood and anxiety disorders, as these were the most common psychiatric disorders among the study population (see Table 2). Plasma concentrations of total IGFBP-3 were significantly higher in patients with anxiety disorder than in patients without anxiety disorder ($t_{54}=-2.305$, $p=0.025$). However, we did not find statistically significant differences in the plasma concentrations of the other neurotrophic factors between patients with and without mood disorder (see Supplementary Tables S5 and S6).

Predictive variables of cognitive impairment and education level in alcohol-abstinent AUD patients. First, to evaluate the predictive models using logistic regression, neurotrophic factors and relevant

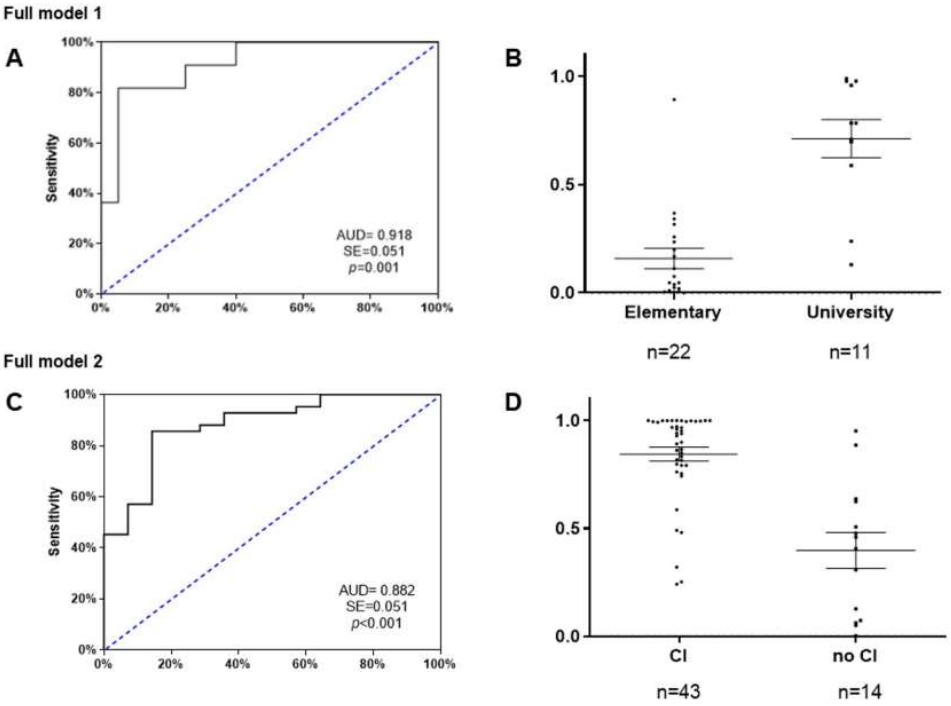

Figure 3. ROC analyses and scatter dots for multivariate predictive of full models of cognitive reserve (top, **A**, **B**) and cognitive impairment (down, **C**, **D**). ROC curves were generated by two binary regression logistic models using neurotrophic factors and alcohol-related variables as predictors following a backward stepwise entry method. (**A**) ROC curve for the full model 1 of cognitive reserve: "plasma concentrations of NT-3", "age at onset of consumption" and "age at development of dependence". (**B**) Scatter plot of the predictive probabilities for full model 1 of cognitive reserve ($U = 18$, $p < 0.001$). (**C**) ROC curve for the full model 2 of cognitive impairment: "plasma concentrations of BDNF", "age at onset of consumption", "length of AUD diagnosis", "severity criteria", and "duration of abstinence". (**D**) Scatter plot of the predictive probabilities for the full model 2 of cognitive impairment ($U = 69$, $p < 0.001$). The lines of the scatterplots are means and standard deviations. *CI* cognitive impairment.

variables associated with alcohol consumption were included according to previous statistical analyses and were introduced using the *backward conditional* method.

Full model 1 was performed to classify patients as having high or low cognitive reserve. The variables "plasma concentrations of NT-3", "age at onset of consumption" and "age at development of dependence" were included in the model in the first step, and all three proved to be good predictor variables. Thus, we observed a new model with a good ability to discriminate between patients with a primary education and those who completed university studies ($X^2 = 4.800$; $p = 0.779$) that was able to explain the variation in the dependent variable in 65.3% of cases, according to the Nagelkerke R2 method. Additionally, the new model had a classification rate of 90.3%, showing a very high sensitivity for the classification of patients with a primary education level (95%) and patients with a university education (81.8%). As shown in Fig. 3A, the ROC curve analysis showed an AUC = 0.918, which indicates high discriminatory power. As shown in Fig. 3B, the scatterplot of the predictive probabilities for patients with primary and university education indicated that the means were significantly different between the two groups ($U = 18$, $p < 0.001$).

Then, a binary logistic regression analysis was used to evaluate the potential for plasma 3-NT concentrations alone to be a good predictor for discriminating between patients with an elementary education and those with a university education. The final model showed a good ability to discriminate between patients with and without cognitive impairment ($X^2 = 1.609$; $p = 0.991$) and was able to explain the variation in the dependent variable in 30.8% of cases, according to the Nagelkerke R2 method. On the other hand, we observed that the model had a classification percentage of 75.8%, showing high sensitivity for the classification of patients with a primary education (86.4%), but not for the classification of patients with a university education (54.5%). The ROC curve analysis showed an AUC = 0.793, which indicates average discriminatory power. The scatterplot of the predictive probabilities for patients with primary and university education indicated that the means were significantly different between the two groups ($U = 50$, $p = 0.006$).

Full model 2 was performed to classify patients with cognitive impairment from those without cognitive impairment. The variables "plasma concentrations of BDNF", "age at onset of consumption", "length of AUD

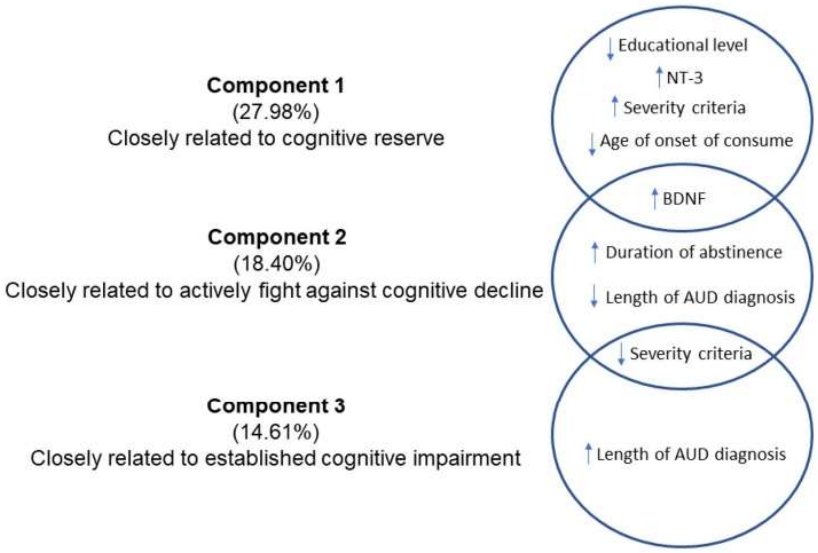

Figure 4. Exploratory principal component analysis in patients with cognitive impairment (n = 43). Three components (factors) together explained 60.98% of the variance associated with cognitive impairment in AUD patients.

diagnosis", "severity criteria", and "duration of abstinence" were included in the model in the first step and all were good predictors. Thus, we created a new model that exhibited a good ability to discriminate between patients with and without cognitive impairment ($X^2 = 6.801$; p = 0.450) and could explain the variation in the dependent variable in 57.8% of cases, according to the Nagelkerke R2 method. Additionally, the new model had a classification percentage of 83.9%, showing average sensitivity for the classification of patients without cognitive impairment (66.7%) and high sensitivity for the classification of patients with cognitive impairment (90.2%). As shown in Fig. 3C, the ROC curve analysis showed an AUC = 0.882, which indicates high discriminatory power. As shown in Fig. 3D, the scatterplot of the predictive probabilities for patients with and without cognitive impairment indicated that the means were significantly different between the two groups (U = 69, p < 0.001).

Subsequently, a binary logistic regression analysis was used to evaluate the potential for plasma concentrations of BDNF to discriminate between patients with cognitive impairment from those without cognitive impairment. The final model showed a good ability to discriminate between patients with and without cognitive impairment ($X^2 = 11.370$; $p = 0.182$) and was able to explain the variation in the dependent variable in 9.1% of cases, according to the Nagelkerke R2 method. Furthermore, the model had a classification percentage of 75.9%, showing high sensitivity for the classification of cognitively impaired patients (97.7%) but not for the classification of nonimpaired patients (13.3%). The ROC curve analysis showed an AUC = 0.731, which indicates average discriminatory power. The scatterplot of predictive probabilities for patients with and without cognitive impairment indicated that the means of the two groups were significantly different (U = 166, $p = 0.009$).

Differential profiles associated with education level and growth factors in alcohol-abstinent AUD patients with and without cognitive impairment. To understand how all of these factors contribute to the differences between groups based on the presence of cognitive decline, a principal components analysis was performed. Three components together explained 60.98% of the variance associated with cognitive impairment in AUD patients (Fig. 4). Component 1 explained 27.98% of the total variance and was closely related to the cognitive reserve of the patients. Education level, NT-3, BDNF, severity of addiction and age at onset of consumption had a high factor load (−0.691, 0.718, 0.346, 0.596, −0.629, respectively). That is, this patient profile corresponds to those with a low educational level who started consuming alcohol early and who currently have high levels of 3-NT and BDNF and severe addiction. Component 2 explained 18.40% of the total variance and was closely associated with patients who are undergoing compensation or actively fighting against cognitive decline. BDNF, the severity of addiction, the duration of current abstinence and the duration since the AUD diagnosis had a high factor load (0.666, −0.366, 0.711, −0.388, respectively). Therefore, this patient profile corresponds to those who currently have higher levels of BDNF, lower severity of addiction, a longer duration of abstinence and a shorter duration of AUD. Lastly, component 3 explained 14.61% of the total variance and was related to patients with established cognitive impairment. The severity of addiction and the duration of consumption had a high factor load (−0.438, 0.858, respectively). In other words, this profile corresponds to patients with a prolonged duration of AUD and a lower severity of addiction at the time of evaluation, possibly as a consequence of an established impairment.

Discussion

Cognitive impairment and neurotrophic factors have been widely described in the scientific literature; however, they have never been linked in an integrated way with cognitive reserve and education level. The main results of this study indicate that (1) education level can act as a protective or risk factor in the onset of AUD and the development of cognitive impairment, (2) cognitive impairment is related to the onset of alcohol consumption and the length of AUD diagnosis throughout the lifespan and not to the patient's psychiatric comorbidity, (3) the cognitive reserve is associated with frontal lobe functions but not with mnesic functions, (4) the plasma concentration of NT-3 is affected by cognitive reserve and can discriminate between patients with a high and low education level, (5) the plasma concentration of BDNF is affected by the state of frontal lobe function and is able to distinguish patients with and without cognitive impairment, (6) the cognitive reserve, NT-3 and BDNF are compensatory mechanisms for brain damage in the early stage of AUD, but not in later phases.

Education level is known to be a robust measure of cognitive reserve that contributes to delaying and smoothing the progress of mild cognitive impairment and other neurodegenerative pathologies[13,16]. In the field of addictions, cognitive reserve has been related to better cognitive performance and lower indicators of neurocognitive disorder[53,54]. The results of our study indicate that patients with a university education were protected against cognitive impairment caused by alcohol consumption throughout life, while patients with a primary education showed a special vulnerability to the occurrence of frontal deficits. Along this line, the consumption of high amounts of alcohol has been associated with worse cognitive performance in patients with low socioeconomic status[55]. Moreover, a longitudinal study indicated that having an education level lower than secondary school and a low-skill occupation are associated with an increased risk of dementia in people who consume alcohol[56]. It is important to mention that the differences in cognitive function found in our results were not due to psychiatric comorbidity, as was also described in another study[57]. In addition, in our study, cognitive reserve, understood as academic achievements, was related to frontal functions, but not with subjective memory failures. In agreement with this, a meta-analysis found that cognitive reserve is related to lateral and medial frontal areas, including anterior cingulate cortex and dorsolateral prefrontal cortex[18]. Thus, cognitive reserve has been associated with some domains of executive functions, such as working memory, verbal fluency and interference[58].

On the other hand, the cognitive reserve of patients with substance use disorders has been related to the severity of the addictive process, the duration of abstinence and cognitive performance[53]. Similarly, our results indicate that patients with a low educational background initiated consumption and developed alcohol dependence early, while the opposite effect was observed for those who had a high education level. We think that the relationship between school dropout and substance use could be due to two factors. First, adolescents who consume alcohol are more likely to drop out of school. This is supported by the McAlaney study, in which the negative consequences most frequently reported by European substance-consuming students were skipping classes, memory problems and poor academic performance[59]. Second, dropping out of school could predispose students to begin consuming alcohol since they are deprived of the protection offered by the educational system and additional years of schooling. Along this line, according to Crum, young people who drop out of high school or college have a higher risk of developing alcohol abuse in adulthood than those who complete college or high school[60]. Regardless of the cause or consequence, repeated alcohol poisoning in adolescence and a lack of cognitive stimulation interfere with brain development, increasing subsequent neuropsychiatric vulnerability[61,62].

Regarding alcohol-related cognitive impairment, the early onset of alcohol consumption seems to be a risk factor for poor subsequent neuropsychological functioning in young adults[63]. Likewise, our results indicate that patients with cognitive impairment were more likely to have started consuming alcohol early and to have had a longer duration of AUD. Thus, they are patients who are at a more advanced stage of the disorder and have the added possibility of experiencing a liver complication in the future. It is interesting to note that patients with alcohol-related cirrhotic disease show greater cognitive deficits and worse brain reserve than patients with non-alcoholic cirrhosis[64]. In addition, patients with cirrhosis who have a high cognitive reserve have a better quality of life, while patients with cirrhosis who have covert encephalopathy show a lower cognitive reserve[65]. On the other hand, the patients in our study without cognitive impairment had a higher number of addiction severity criteria and a shorter duration of abstinence at the time of evaluation, indicating that they were patients who were actively fighting the disorder. This finding demonstrates that cognitive impairment is not associated with the amount of alcohol consumption but rather with the onset of consumption and the duration of AUD throughout life[63]. In addition, this result indicates that cognitive function does not improve with the duration of abstinence and may indicate permanent damage to cognitive abilities in alcohol-consuming patients, as indicated by other studies[35,66,67].

On the other hand, clinical and preclinical studies have widely described that alcohol produces a proinflammatory condition in the central nervous system[26,68]. Microglia respond to alcohol through TLR4, which activates signaling cascades, including the NF-KB and MAPK pathways, which induce the activation of proinflammatory mediators[29,69], to the detriment of trophic signaling and cognition[28,70]. Previously, our team found decreases in plasma concentrations of NT-3 and BDNF and an association between frontal functions and circulating BDNF in patients with AUD[46,50]. In light of our results, we can say that the decrease in the BDNF and 3-NT levels of AUD patients does not interfere with frontal function in those with higher education, probably because cognitive reserve compensates for these biochemical deficits. However, the decrease in the levels of BDNF and 3-NT seems to trigger cognitive decline in patients with low education levels because their low cognitive reserve prevents them from compensating for organ damage derived from alcohol consumption. The fact that 3-NT levels are higher in patients with a low education level than in those with a high education level could reveal the failed attempt of the system to compensate for the brain damage with neurogenesis instead of implementing more effective compensatory mechanisms that depend on a higher cognitive reserve.

In terms of growth factors, BDNF seems to be a candidate for describing the neurobiological basis of cognitive reserve[71]. Physical activity, social interactions, cognitive stimulation, a high education level and an enriching environment have been associated with increases in BDNF levels and a lower risk of dementia[44,72–75]. In contrast, Val66 Met polymorphism is known to prevent the release of mature BDNF, disrupt cognitive functioning[76–78] and interfere with the protective effect of cognitive reserve on executive functions[79]. However, it seems that the scientific literature has underestimated the role of 3-NT in cognitive processes in adult life.

In our study, 3-NT and BDNF were shown to be robust factors of brain damage and cognitive reserve, respectively, that allowed us to discriminate very clearly between cognitively impaired and non-impaired patients and between patients with high and low cognitive reserve. In addition, we have shown the compensatory role that these two neurotrophins have on cognitive decline in the early phase of AUD. Thus, in a subgroup of patients, we observed that NT-3 and BDNF signaling is initiated to compensate for the damage caused by alcohol in patients with a low cognitive reserve who started drinking early. Second, in another subset of patients, we showed that the compensatory signal of BDNF is also activated independently of the cognitive reserve in the early stage of AUD. In contrast, in a third subset of patients, we observed that neurotrophic signaling and the protective effect of cognitive reserve disappear in the advanced phase of AUD. Therefore, these findings likely indicate that BDNF levels decrease when organ damage is already established. In line with our results, there is an emerging line of research that supports the role of the cognitive reserve and the BDNF/TrkB signaling pathway as compensatory responses that delay symptomatology in the early stage of Alzheimer's disease but cannot prevent neurodegeneration in more advanced phases[16,80–82]. Although BDNF has not been shown to be able to differentiate among different neurodegenerative diseases[83], it does seem to be a good predictor of the severity and progression of cognitive impairment[74,84].

In conclusion, educational history and a simple cognitive evaluation, together with the measurement of BDNF, allowed us to identify patients who are at risk of cognitive impairment and differentiate them from those who are already cognitively impaired, stratifying them in a very clear way. Such information allows interventions to be personalized according to the stage of the neuropathology, such as by recommending cognitive and physical stimulation for less affected people or establishing palliative treatment in more severe cases. Additionally, this knowledge should focus attention on early care measures in anticipation of future needs since school dropout and alcohol consumption lead to the impairment of cognitive abilities in adult life.

Limitations and future prospects. This study has a number of limitations that future research should take into account. The study has a small sample of patients and lacks significant representation of the female population, which prevents the investigation of gender/sex differences in educational level, cognitive impairment and neurotrophic factors. Finally, the study did not include another relevant growth factor, Nerve Growth Factor (NGF) that might be accounting for the cognitive impairment described in AUD patients, and that should be analyzed in future studies.

Methods

Recruitment and screening of participants. The study was conducted based on a cohort of Addictive Disorders Network (RTA, for its initials in Spanish) patients with AUD recruited from 2016 to date. We relied on a database with a total of 262 alcohol-abstinent patients in outpatient treatment, 148 of whom had undergone measurements of neurotrophic factors in blood plasma. Of these 148 patients, we included 58 patients who had undergone a brief neuropsychological evaluation using the Frontal Assessment Battery (FAB) and Memory Failures of Everyday questionnaire (MFE), which will be described below. The patients were recruited from the Psychiatry Service of the *12 de Octubre University Hospital* (Madrid, Spain) and the *Provincial Drug Addiction Center* (Málaga, Spain).

To be eligible for the study, subjects has to meet eligibility criteria based on (A) inclusion criteria: age ≥ 18 years up to 65 years of age, lifetime AUD, and at least 2 weeks of abstinence before testing confirmed by repeated negative breathalyzer test; (B) exclusion criteria: personal history of chronic diseases (e.g. cardiovascular, respiratory, renal, hepatic, neurological or endocrinological diseases), personal history of autoimmune disorders, cancer, presence of chronic viral infectious diseases (VIH, HB, HC), incapacitating cognitive alterations to complete psychiatric interview and neuropsychological evaluation, and pregnancy for female participants.

Ethical declaration. Written informed consent was obtained from each participant after a complete description of the study was provided. All participants had the opportunity to discuss any questions or problems. The study and the protocols for recruitment were approved by the Ethics Committee of the Regional University Hospital of Málaga in accordance with the Ethical Principles for Medical Research involving Human Subjects adopted in the Declaration of Helsinki by the World Medical Association (64th General Assembly of the WMA, Fortaleza, Brazil, October 2013) and Recommendation no. R (97) 5 of the Committee of Ministers to the Member States on the protection of medical data (1997), the Spanish law on data protection [Regulation (EU) 2016/679 of the European Parliament and of the Council of April 27, 2016 on the protection of natural persons with respect to the processing of personal data and the free circulation of such data, and which repeals Directive 95/46/EC (General Data Protection Regulation)]. All collected data received code numbers to maintain privacy and confidentiality.

Psychiatric and neuropsychological evaluation. The Spanish version of the PRISM (Psychiatric Research Interview for Substance and Mental Diseases) diagnostic interview was used for the evaluation of substance use disorders and other psychiatric disorders in accordance with to the criteria of the DSM-IV-TR (Diagnostic and Statistical Manual of Disorders Mental, 4th edition, text review). The PRISM is a semistructured

interview with good psychometric properties for the evaluation of substance use disorders and the main psychiatric disorders comorbid with substance use[85,86].

The neuropsychological evaluation was performed using two different tests that have proven reliability and good psychometric properties: the Spanish version of the FAB, which was used to diagnose frontal dysfunction[51] and the MFE, which was used to evaluate daily memory failures[52]. The total FAB score ranges from 0 to 18, and the test evaluates the subdomains prehension behavior, go-no go, conflicting instructions, lexical fluency and Luria's motor series. A cutoff score lower than 16 differentiates normal frontal function from mild deficits, and a score below the cutoff score of 13 differentiates mild and severe frontal lobe dysfunction[51]. The MFE questionnaire consists of 30 items and is useful for evaluating memory failures in daily life. Cognitive complaint scores of less than eight points correspond to optimal memory functioning, scores between 8 and 35 points are equivalent to normal functioning with insignificant memory failures, scores between 36 and 50 indicate deterioration in memory function with some impact on everyday life, and scores above 50 points correspond to moderate or severe deterioration with substantial impact on daily functioning[52].

Obtaining plasma samples. Blood samples were obtained in the morning after an 8- to 12-h fast (before psychiatric interviews). Venous blood was extracted into 10-ml K2 EDTA tubes (BD, Franklin Lakes, NJ, USA) and immediately processed to obtain plasma. Blood samples were centrifuged at 2200×*g* for 15 min (4 °C) and analyzed individually for infectious diseases using three rapid commercial tests for HIV, hepatitis B and hepatitis C (Strasburg, Cedex, France). Finally, the plasma samples were aliquoted, recorded and stored individually at − 80 °C until further analysis.

Analysis of neurotrophic factors. Plasma levels of BDNF, IGF-2 and NT-3 were determined using different enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions: the human BDNF SimpleStep® ELISA kit (# ab212166, Abcam, Cambridge, UK), the Quantikine Human IGF-2 ELISA Kit (# DG200, R&D Systems, Minneapolis, MN, USA) and the NT-3 ELISA Kit (# EHNTF3, Thermo Fisher Scientific, Alcobendas, Madrid, Spain). To perform the ELISA protocols, we used 50 µL of plasma, as previously described[50]. Plasma concentrations of IGF-1 and IGFBP-3 were estimated by double antibody radioimmunoassay, as described in[39]. Plasma fractions were incubated with 125I-IGF-1 at 4 °C for 24 h with IGF-1 antiserum (UB2-495). The plasma concentration of IGFBP-3 was determined in duplicate by RIA using a commercially available kit (Mediagnost GmbH, Reutlinger, Germany) according to the manufacturer's instructions. IGFBP-3 concentrations were expressed as µg/mL. A calibration curve and internal controls were included in each test.

Statistical analysis. All data in the tables are expressed as numbers, percentage of subjects [N (%)], means and standard deviations (SD). The significance of the differences for qualitative variables and normal continuous variables was determined using Fisher's exact test (chi-square) and Student's t test, respectively.

Statistical analyses of the FAB and MMSE cognitive scores and concentrations of BDNF, 3-NT, IGF-1, IGF-2 and IGFBP-3 were performed using a univariate analysis of covariance (ANCOVA) to determine the relative effects of education level when covariates such as age and body mass index (BMI) were controlled. Post hoc tests for multiple comparisons were performed using the Bonferroni correction.

Before the continuous variables were included in these analyses, the assumptions of normality and homoscedasticity were confirmed. The normal distribution of the variables was evaluated using the Shapiro–Wilk test. Logarithmic (10) transformations of the variables with nonnormal distribution were used to preserve the parametric assumptions of distributions with positive bias and estimated marginal means [95% confidence interval (95% CI)]. The equality of variances was tested with the Levene test, using Welch's T in cases of noncompliance.

To identify the variables that were able to discriminate between impaired and nonimpaired patients and between patients with an elementary and a university education, a binary logistic regression analysis was performed using Pearson's chi-square test (χ^2), and the results satisfied the Hosmer–Lemeshow test. Sociodemographic variables, variables related to cocaine use patterns and psychiatric comorbidity variables were included in the equation.

Lastly, an exploratory factor analysis with varimax rotation and bivariate relationships (correlation) was performed to determine the different profiles of alcohol-abstinent patients with cognitive decline. Only variables with a factor load of at least 0.3 (i.e., those that share at least 10% of the variance with a factor) were used for the interpretation. A p value less than 0.05 was considered statistically significant.

Statistical analyses were performed using GraphPad Prism version 5.04 and IBM SPSS Statistical version 22 (IBM, Armonk, NY, USA). A value of $p < 0.05$ was considered statistically significant.

Data availability

The data that support the findings of this study are available on reasonable request from the corresponding author.

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Conclusiones parciales del Estudio 1:

- El nivel educativo puede actuar como un factor protector o de riesgo en la aparición de TUA y el desarrollo de deterioro cognitivo.
- El deterioro cognitivo está relacionado con el inicio del consumo de alcohol y la duración del diagnóstico de TUA a lo largo de la vida y no con la comorbilidad psiquiátrica del paciente.
- La reserva cognitiva, medida como nivel de estudios alcanzado, se asocia con funciones del lóbulo frontal pero no con funciones mnésicas.
- La concentración plasmática de NT-3 se ve afectada por la RC y puede discriminar entre pacientes con un nivel educativo alto y bajo.
- La concentración plasmática de BDNF se ve afectada por el estado de la función del lóbulo frontal y es capaz de distinguir pacientes con y sin deterioro cognitivo.
- La RC, NT-3 y BDNF son mecanismos compensadores del daño cerebral en la etapa temprana de TUA, pero no en fases posteriores.

4.2. Estudio 2:

Influencia del género y la educación en consumidores de cocaína en una cohorte de pacientes ambulatorios en España

"Influence of gender and education on cocaine users in an outpatient cohort in Spain"

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Influence of gender and education on cocaine users in an outpatient cohort in Spain

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Gender significantly influences sociodemographic, medical, psychiatric and addiction variables in cocaine outpatients. Educational level may be a protective factor showing less severe addictive disorders, longer abstinence periods, and better cognitive performance. The aim was to estimate gender-based differences and the influence of educational level on the clinical variables associated with cocaine use disorder (CUD). A total of 300 cocaine-consuming patients undergoing treatments were recruited and assessed using the Psychiatric Research Interview for Substance and Mental Diseases according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. Women developed CUD later but exhibited more consumption of anxiolytics, prevalence of anxiety disorders, eating disorders, and major depressive disorders. Alcohol and cannabis use disorders were more frequent in men. A predictive model was created and identified three psychiatric variables with good prognosis for distinguishing between women and men. Principal component analysis helped to describe the different profile types of men and women who had sought treatment. Low educational levels seemed to be a risk factor for the onset, development, and duration of CUD in both genders. Women and men exhibited different clinical characteristics that should be taken into account when designing therapeutic policies. The educational level plays a protective/risk role in the onset, development and progression of CUD, thus prolonging the years of compulsory education and implementing cognitive rehabilitation programmes could be useful.

Cocaine is a psychoactive substance whose consumption causes a strong health, social, and economic impact[1,2]. This substance is the most widely used illegal stimulant in Europe. In recent years, Spain has occupied the second place in the EU with respect to cocaine consumption[1].

In Spain, 10.9% of adults (15–64 years) have used cocaine throughout their lives, 2.5% in the last year, and 1.1% in the last month[1,3]. Particularly, in Andalusia (Southern of Spain), the prevalence of admissions to treatment for cocaine use has increased from 4591 in 2014 to 5827 patients in 2018[4].

Drug use has always been more prevalent in men than in women. This fact resulted from the male social and cultural role that allowed access much easier and the strong stigma around consumption in women[5,6]. At present, equal rights have allowed a rapid expansion of the consumption of different addictive substances among young women[6], a reality that has already been reflected in the reports of school surveys.

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Regarding to gender (considered as the sex [man or woman] reported by the patient when interviewed) and prevalence of psychoactive substance use in the last year, drug consumption is more widespread in men than in women except for hypnosedatives (62.4%) and opioid analgesics (56.6%)[3,7]. In the last years there was an increase of cocaine use for both genders, although is still more pronounced among men[7]. However, women who use cocaine suffer more social discrimination, have lower educational levels, higher unemployment rates, worse socioeconomic status, and worse health conditions. Instead, men exhibit greater prevalence of legal or criminal problems[8–10].

In recent years, studies have addressed gender-based differences in patients with CUD[5,8]. Prevalence, use onset, progression of the disorder, relapses, and response to treatment have been found to vary between women and men[11,12]. Studies have indicated that women have lower rates of cocaine use[1,8,13] and attend treatment centres to a lesser extent[1,14]. Furthermore, despite the fact that women initiate cocaine use later[5,15] they are as likely as men to develop dependence after the first use[16] and exhibit greater severity of addiction[8,14]. Women experience accelerated progression from the onset of consumption to the pathological use of cocaine[5,9]. This earlier process, called 'telescoping', has also been observed with other addictive substances such as alcohol, cannabis, and opiates[5]. In addition, women have higher relapse rates after stressful and/or depressive events[17].

Taking into consideration the factors that contribute to worse CUD prognoses, it is worth mentioning that women exhibit greater predisposition to certain psychiatric comorbidities[5,10]. Specifically, women who use cocaine experience more anxiety, mood, and eating disorders[8,14,18]. On the other hand, antisocial personality disorders and alcohol and cannabis use disorders are more frequent among men who use cocaine, with earlier onset and more years of use than among women[19,20].

Moreover, it is important to point out the occurrence of biases in the legal prescription of psychotropic medication to women, especially anxiolytics[21]. If we consider that such medication for primary psychiatric disorders loses effectiveness as drugs are used in combination, the management of these comorbid patients becomes much more difficult. For example, the efficacy of antidepressants is reduced by concomitant substance use[22]. Furthermore, benzodiazepines are prescribed over long periods of time, even though their prolonged use is not recommended due to the risk of dependence, which contributes to the appearance of disorders derived from the use of anxiolytics disguised by a legal route[21].

In addition, some authors have indicated that the development of skills and experiences in patients with substance use disorders—such as academic achievements, occupational level, leisure activities, and social support—have been related to less serious addictive disorders, longer periods of abstinence, and better cognitive performances[23]. However, very few studies have explored the effects of these variables on cocaine-use onset and the development of CUD. One study indicated that cocaine-use patterns in European students were strongly related to socioeconomic status and educational levels, as well as school absenteeism and low level of leisure reading[24]. In addition, the most frequent negative consequences exhibited by these students who used substances of abuse were being absent from classes, memory problems, and low academic performance[25]. In another study conducted with an adolescent population, the authors observed that educational achievements had very important weight in the progression towards substance use disorders[26]. Furthermore, gender discrimination has been consistently associated with the use of illicit drugs and substance use disorders among women in the US, with those with an educational level below secondary occupying a position of greater risk[27].

In light of the abovementioned factors, and given the need to promote differential therapeutic approaches between women and men, as well as prevention measures through educational level, the main objectives of the present study were to: (1) estimate the differences between women and men with respect to sociodemographic characteristics, other substances of abuse and psychiatric comorbidities; (2) determine the effect of gender and educational level on cocaine-related variables; (3) create a predictive model capable of discriminating between women and men, based on the aforementioned variables that can allow differential diagnoses; and (4) describe the different gender-based profiles in patients treated for cocaine use.

Method

Study design and cohort. This is a retrospective, descriptive and observational study conducted with a cohort of 300 cocaine users undergoing outpatient treatment centres divided into two groups according to gender (women *vs.* men). Abstinent patients were recruited in different drug addiction outpatient treatment centres (Málaga, Spain).

The inclusion criteria to be eligible for the present study were individuals aged over 18 years; cocaine users in the abstinence phase; being under outpatient treatment; and willingness to participate by signing an informed consent form. The chosen patients were evaluated to diagnose lifetime CUD and others psychiatric comorbidities based on DSM-IV-TR. Exclusion criteria included the presence of severe cognitive alterations and being in an acute psychotic episode in the active phase, which would not allow the normal development of the clinical assessment.

The present study fits within the framework of projects promoted by the Red de Trastornos Adictivos [RTA] (Addictive Disorders Network), an entity financed by the Instituto de Salud Carlos III (ISCIII), belonging to the Ministerio de Ciencia e Innovación of Spain. The ethical aspects of the core project (Proteomics of Cocaine Addiction: Central and Peripheral Biomarkers of Addiction) were approved by the Ethics and Clinical Research Committee of the Regional University Hospital of Malaga, respecting the ethical principles for medical research on human subjects adopted in the World Medical Association Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, 2013). The assessment process was carried out by a team of clinical psychologists who had specialised and accredited training in psychiatric assessments.

Measuring instruments. The Spanish version of the Psychiatric Research Interview for Substance and Mental Diseases (PRISM) was used to collect sociodemographic data and assess psychiatric disorders according to DSM-IV-TR criteria. PRISM is a semi-structured interview with good psychometric properties in the evaluation of substance use disorders and in the main comorbid psychiatric disorders related to the substance use population (Kappa coefficient between 0.66 and 1.00)[28]. First module of questions assesses the history of substance consumption, and second module assesses twenty Axis I and two Axis II disorders that are more prevalent in this population. One of the most important characteristics of this instrument is that it allows differentiating primary or independent mental disorders from substance-induced disorders[29,30]. The unidimensionality of the DSM-IV-TR criteria for CUD was used to determine cocaine trait severity combining the seven dependence criteria (for diagnosis of dependence three or more co-occurring symptoms in a 12-month period are required) and the four abuse criteria (one symptom is necessary for diagnosis of abuse).

Procedure. A member of the therapeutic team collaborated in each outpatient treatment centre for recruitment of the participants. This person was in charge of informing about the existence of the study and inviting patients who had requested treatment at some point to participate. If they met the established inclusion criteria, the patients were referred to the team of clinical psychologists. In this way, the latter were in charge of summoning the patients and travelling through the different outpatient treatment centres in the Province of Malaga, Spain, where the clinical assessments were performed once the informed consent form had been signed by the participants.

The psychiatric assessments were performed in the same morning and could last between two and three hours. Finally, each interview was recorded in a database designed for the study. The interviews were conducted between 2010 and 2020.

Study variables. The variables of the present study were: (a) Sociodemographic variables: age; marital status; number of children; educational level; occupational status; and cell/prison; (b) Medical, therapeutic and psychopharmacological treatment variables: chronic medical problems; psychiatric/psychological support; attendance at twelve-step groups; psychotropic medications (last 12 months); anxiolytics; antidepressants; antipsychotics; abstinence maintenance treatment; and disulfiram; (c) Psychiatric comorbidity variables: total psychiatric comorbidity; mood disorders; major depressive disorder; dysthymia; manic episode; hypomanic episode; cyclothymia; anxiety disorders; generalised anxiety disorder; obsessive compulsive disorder; post-traumatic stress disorder; seizure panic disorder; specific phobias; social phobia; psychotic disorders; schizophrenia; schizophreniform disorder; unspecified psychotic disorder; brief psychotic disorder; eating disorders; anorexia; bulimia; personality disorders; borderline personality disorder; antisocial personality disorder; attention-deficit hyperactivity disorder; and (d) Cocaine-related variables: severity criteria; age at onset of use; age at dependence development; length of abstinence; number of abstinences; duration of CUD; alcohol use disorder; cannabis use disorder; and sedatives use disorder.

Statistical analysis. The results were analysed using the statistical programme SPSS version 19.0 (IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp). All data in the tables are expressed as numbers and percentage of subjects [N (%)] or means and standard deviations (SD). First, the differences in the qualitative variables were determined using Fisher's exact test [Chi-square test (χ^2)]. Despite the small number of women in the study, we analysed gender differences in sociodemographic, psychiatric comorbidity and cocaine-related variables using different chi-square test according to the number of categories and the sample size:

- Chi-square when there was more than two categories and a high sample size for each group [N > 5(%)].
- Chi-square by trend or linear by linear association when there was more than two categories and a small sample size for each group [N < 5 (%)].
- Fisher's exact test for two categories and a small sample size.

The normal distribution of the variables was assessed using Lilliefors corrected Kolmogorov–Smirnov test. As the continuous variables of the study did not meet the assumption of normality, statistical analyses were performed using non-parametric Mann–Whitney U test for comparisons between two groups, and Kruskal–Wallis test for comparisons of more than two groups. For *post-hoc* analyses we performed Dunn's multiple comparison test.

To determine the variables that were able to discriminate between women and men, a binary logistic regression analysis was performed using Pearson's Chi-square (χ^2) test, meeting the Hosmer–Lemeshow test. We assayed multicollinearity by examining Tolerance and Variance Inflation Factor (VIF). The cut off value for Tolerance was > 0.10 and < 10 for VIF. Sociodemographic variables, variables related to cocaine use patterns and psychiatric comorbidity variables were included in the equation. Finally, exploratory factor analysis with varimax rotation and bivariate relationships (correlation) was performed to determine the different profiles of patients who attended outpatient treatment for cocaine use, differentiated by gender. Only variables with a factorial load of at least 0.3 (sharing at least 10% of the variance with a factor) were used for interpretation. A p value less than 0.05 was considered statistically significant.

The discriminative power of the logistic model and the thresholds of certain variables were evaluated by Receiver Operating Characteristics (ROC) analysis considering the Area Under the Curve (AUC). A p value less than 0.05 was considered statistically significant.

Variable		Total N = 300	Men N = 254	Women N = 46	*p* Value
Age [media (SD)]	Years	35.89 (8.95)	35.60 (9.07)	37.52 (8.19)	0.072[a]
Marital status [N (%)]	Single Married/cohabiting Divorced/separated Widower	131 (43.70) 106 (35.30) 61 (20.30) 2 (0.70)	110 (43.30) 88 (34.60) 56 (22.00) –	21 (45.70) 18 (39.10) 5 (10.90) 2 (4.30)	0.702[b]
Children [mean (SD)]	Number	1.02 (1.26)	1 (1.26)	1.13 (1.22)	0.412[b]
Educational level [N (%)]	Primary/elementary Secondary University	72 (24.00) 195 (65.00) 33 (11.00)	60 (23.60) 167 (65.70) 27(10.60)	12 (26.10) 28 (60.90) 6 (12.00)	0.800[b]
Occupational status [N (%)]	Employed Medical sick leave Unemployed Retired Homework	105 (35.00) 21 (7.00) 160 (53.30) 11 (3.70) 3 (1.00)	89 (35.00) 20 (7.90) 134 (52.80) 10 (3.90) 1 (0.40)	16 (34.80) 1 (2.20) 26 (56.50) 1 (2.20) 2 (4.30)	0.450[b]
Prison/detained [N (%)]	Yes No	125 (41.70) 175 (58.30)	111 (43.7) 143 (56.3)	14 (30.4) 32 (69.6)	0.105[b]

Table 1. Sociodemographic characteristics in total, men, and women cocaine users cohort. *N* number of patients, *SD* standard deviation, *%* percentage, *BMI* Body Mass Index. [a]*P*-value of the the Mann–Whitney U test. [b]*P*-value of the Chi-Square test.

Results

Sociodemographic characteristics in cocaine users cohort. The description of the sociodemographic variables of the sample composed of 300 patients is illustrated in Table 1. Among patients who attended outpatient treatment for cocaine use, the 84.7% were men and the mean age was 35.6 years (SD = 9.1); whereas the 15.3% were women, with a mean age of 37.5 years (SD = 8.2). We did not find statistically significant differences in sociodemographic variables when comparing women and men.

In addition, the 90.7% of the total sample were patients diagnosed with CUD while the 9.3% were patients who did not meet the diagnosis of CUD according to DSM-IV-TR.

Medical, therapeutic, and psychopharmacological variables according to gender in cocaine users cohort. We performed an analysis to assess gender bias in prescribed psychotropic drugs. There were significant differences ($\chi^2 = 11.63$, $p = 0.001$) in the percentage of total consumption of psychotropic medication between women and men (84.1% vs. 56.9%). These differences were especially relevant for consumption of anxiolytics ($\chi^2 = 8.53$, $p = 0.005$), since women took them more compared to men (63.3% vs 39.9%) (Supplementary Table 1S).

Comorbid psychiatric disorders in CUD patients according to gender. We investigated the influence of gender in the psychiatric comorbidity associated with CUD. There were significant differences in the prevalence of anxiety disorders ($\chi^2 = 15.17$, $p < 0.001$) and eating disorders ($\chi^2 = 10.65$, $p = 0.007$) between genders being more prevalent in women than in men (45.5% vs 18.5% and 11.4% vs 1.8%, respectively) (Supplementary Table 2S).

The variables related to specific comorbid psychiatric disorders are illustrated in Table 2. We observed significant gender differences for generalised anxiety disorder ($\chi^2 = 7.88$, $p = 0.0013$) and post-traumatic stress disorder ($\chi^2 = 16.96$, $p < 0.001$) being more prevalent in women than in men (22.7% vs 8.3% and 25% vs 5.7%, respectively). Similarly, we found significant differences in the diagnosis of anorexia ($\chi^2 = 20.946$, $p = 0.001$) and bulimia ($\chi^2 = 3.74$, $p = 0.021$) being more frequent in women compared to men (9.1% vs 1.8% and 6.8% vs 0%, respectively). On the other hand, there were significant gender differences in the presence of comorbid alcohol use disorder ($\chi^2 = 8.77$, $p = 0.004$) and cannabis use disorder ($\chi^2 = 7.16$ $p = 0.022$), being more prevalent in men than women (60.5% vs. 36.4% and 28.2 vs 11.4%, respectively).

Regarding the type of substance use disorder, we found statistically significant gender differences according to the type of disorder (primary, induced or both) with respect to major depressive disorder ($\chi^2 = 3.91$, $p = 0.048$). Specifically, women had more major depressive disorders of both types (induced and primary) than men (33.30 vs 2.30%, $p = 0.012$) (Supplementary Table 3S).

Impact of gender and educational level on cocaine-related variables in CUD patients. We analysed the influence of gender and educational level in the variables associated with cocaine use. As shown in Table 3, we observed the effect of gender for the variable 'age at development of dependence' (U = 3463.50, $p = 0.010$, $\eta 2 = 0.025$). Women developed cocaine dependence 2.5 years later than men, at the age of 28.1 years for women and 25.6 years for men.

As shown in Table 4, we found an effect of educational level for the variables 'age at the onset of use' (H = 8.23, $p = 0.016$), 'age at development of dependence' (H = 14.20, $p = 0.001$) and 'duration of CUD' (H = 7.84, $p = 0.020$). CUD patients with primary education started cocaine use 6.22 years earlier than patients with university studies (U = 172, $p = 0.006$, $\eta 2 = 0.124$). Similarly, CUD patients with primary education developed cocaine dependence 6.52 years earlier than those with university education (U = 445, $p < 0.001$, $\eta 2 = 0.134$) and patients with secondary educational had cocaine dependence 4.64 years earlier than those with university education (U = 1334, $p = 0.003$,

Variable			Cocaine use disorder			
			Total N = 272	Men N = 228	Women N = 44	*p* Value
Mood disorders	Major depressive disorder [N (%)]	Yes No	73 (26.80) 198 (72.80)	61 (26.80) 166 (72.80)	12 (27.30) 32 (72.70)	<0.999
	Dysthymia [N (%)]	Yes No	10 (3.70) 261 (96)	8 (3.50) 219 (96.10)	2 (4.50) 42 (95.50)	0.667
	Manic episode [N (%)]	Yes No	5 (1.80) 265 (97.40)	5 (2.20) 221 (96.90)	– 44 (100)	<0.999
	Hypomanic episode [N (%)]	Yes No	4 (1.50) 268 (98.50)	3 (1.30) 225 (98.70)	1 (2.30) 225 (98.70)	0.508
	Cyclothymia [N (%)]	Yes No	4 (1.50) 266 (97.80)	2 (0.90) 224 (98.20)	2 (4.50) 42 (95.5)	0.126
Anxiety disorders	Generalized anxiety disorder [N (%)]	Yes No	29 (10.70) 241 (88.60)	19 (8.30) 207 (90.80)	10 (22.70) 34 (77.30)	**0.013**
	Obsessive compulsive disorder [N (%)]	Yes No	3 (1.10) 267 (98.20)	2 (0.90) 224 (98.20)	1 (2.30) 43 (97.70)	0.415
	Post traumatic stress disorder [N (%)]	Yes No	24 (8.90) 247 (90.80)	13 (5.70) 214 (93.90)	11 (25.00) 33 (75.00)	**< 0.001**
	Panic attack [N (%)]	Yes No	11 (4) 260 (95.60)	8 (3.50) 219 (96.10)	3 (6.80) 41 (93.20)	0.257
	Specific phobias [N (%)]	Yes No	8 (2.90) 263 (96.70)	5 82.20) 222 (97.40)	3 (6.80) 41 (93.20)	0.124
	Social phobias [N (%)]	Yes No	1 (0.40) 271 (99.60)	1 (0.40) 227 (99.60)	– 44 (100.00)	<0.999
Psychotic disorders	Schizophrenia [N (%)]	Yes No	– 272 (100)	– 228 (100)	– 44 (100)	–
	Schizophreniform disorder [N (%)]	Yes No	3 (1.10) 269 (98.90)	2 (0.90) 226 (99.10)	1 (2.30) 43 (97.70)	0.412
	Delusional disorder [N (%)]	Yes No	4 (1.50) 268 (98.5)	3 (1.30) 225 (98.70)	1 (2.30) 43 (97.70)	0.508
	Schizoaffective disorder [N (%)]	Yes No	3 (1.10) 269 (98.90)	2 (0.90) 226 (99.10)	1 (2.30) 43 (97.70)	0.412
	Unspecified psychotic disorder [N (%)]	Yes No	5 (1.80) 267 /98.20)	4 (1.80) 224 (98.2)	1 (2.30) 43 (97.70)	0.589
	Brief Psychotic Disorder [N (%)]	Yes No	14 (5.10) 258 (94.90)	13 (5.70) 215 (94.30)	1 (2.30) 43 (97.70)	0.703
Eating disorders	Anorexia [N (%)]	Yes No	4 (1.50) 267 (98.20)	– 228 (100)	4 (9.10) 40 (90.90)	**0.001**
	Bulimia [N (%)]	Yes No	7 (2.60) 264 (97.10)	4 (1.80) 223 (97.80)	3 (6.80) 41 (93.20)	**0.021**
Personality disorders	Antisocial personality disorder [N (%)]	Yes No	60 (22.10) 210 (77. 20)	53 (23.50) 173 (76.50)	7 (15.90) 37 (84.10)	0.325
	Borderline personality disorder [N (%)]	Yes No	52 (19.10) 218 (80.10)	41 (18) 185 (81.10)	11 (25) 33 (75)	0.300
Other substance use disorders	Alcohol use disorder [N (%)]	Yes No	154 (56.60) 118 (43.40)	138 (60.50) 90 (39.50)	16 (36.40) 28 (63.60)	**0.004**
	Cannabis use disorder [N (%)]	Yes No	68 (25.10) 203 (74.90)	64 (28.20) 163 (71.80)	5 (11.40) 39 (88.60)	**0.022**
	Sedative use disorder [N (%)]	Yes No	28 (10.30) 244 (89.70)	24 (10.50) 204 (89.50)	4 (9.10) 40 (90.90)	0.999

Table 2. Prevalence of psychiatric comorbidity of total, men, and women with CUD (DSM-IV-TR). *N* number of patients, % percentage. *P*-value of the Chi-Square test. Significant *P*-value in bold.

$\eta 2 = 0.046$). Patients with primary education presented 3.47 years longer of CUD than those with secondary ($U = 4079.50$, $p = 0.008$, $\eta 2 = 0.031$).

Additionally, we explored multiple paired comparisons between gender and education for cocaine use patterns. Regarding the woman group, we found significant differences in 'age at the onset of use' ($H = 8.81$, $p = 0.012$). Women with primary education started cocaine use 7.5 years earlier than those with secondary education ($U = 24$, $p = 0.007$, $\eta 2 = 0.256$). Among men group, we observed significant differences in 'age at the onset of use' ($H = 11.22$, $p = 0.004$) and 'age at development of dependence' ($H = 14.95$, $p = 0.001$). Thus, men with primary education started cocaine use three years earlier than those with secondary education ($U = 1752.50$, $p = 0.005$, $\eta 2 = 0.049$), and 6.6 years earlier than those who had attended university ($U = 106$, $p = 0.007$, $\eta 2 = 0.142$). Similarly, men with primary education had cocaine dependence three years earlier than those with secondary educational ($U = 3089.50$, $p = 0.008$, $\eta 2 = 0.036$) and 7 years earlier than those with university education ($U = 281.50$, $p = 0.001$, $\eta 2 = 0.148$). Moreover, men with secondary education had cocaine dependence 4.33 years earlier than those with university education ($U = 941.50$, $p = 0.013$, $\eta 2 = 0.038$). Among those with primary education, men started

Variable		Cocaine use disorder N = 272		
		Men N = 228	Women N = 44	*p* Value
Severity criteria [mean (95% CI)]	Criteria (0–11)	8.09 (7.68–8.49)	7.94 (7.10–8.79)	0.174
Age at onset of use [mean (SD)]	Years	20.13 (6.99)	20.45 (7.12)	0.454
Age at dependence development [mean (SD)]	Years	25.55 (7.49)	28.14 (6.31)	**0.010**
Length of abstinence [mean (SD)]	Days	192.34 (661.03)	147.36 (216.20)	0.488
Number of abstinences [mean (SD)]	Number	1.51 (0.96)	1.34 (0.94)	0.220
Duration of CUD [mean (SD)]	Years	7.72 (6.84)	7.46 (5.81)	< 0.999
Telescoping effect [mean (SD)]	Years	5.66 (5.48)	6.19 (6.23)	0.656

Table 3. Differences in cocaine consumption patterns in patients with CUD according to gender. % percentage, *CI* confidence interval, *SD* standard deviation, *CUD* cocaine use disorder. *P* value for Mann–Whitney U test. Significant *P*-value in bold.

Variable		Cocaine use disorder N = 272			
		Primary N = 69	Secondary N = 178	University N = 25	*p* Value
Severity criteria [mean (95% CI)]	Criteria (0–11)	8.03 (7.32–8.74)	8.17 (7.70–8.63)	7.85 (6.73–8.97)	0.473
Age at onset of use [mean (SD)]	Years	18.33 (5.76)	20.35 (7.04)	24.93 (8.28)	**0.016**
Age at dependence development [mean (SD)]	Years	24.12 (6.88)	26.01 (7.20)	30.64 (7.88)	**0.001**
Length of abstinence [mean (SD)]	Days	199.42 (454.97)	173.56 (681.67)	219.75 (316.75)	0.127
Number of abstinences [mean (SD)]	Number	1.40 (0.89)	1.44 (0.933)	2.00 (1.25)	0.074
Duration of CUD [mean (SD)]	Years	10.28 (9.04)	6.81 (5.34)	6.52 (5.52)	**0.020**
Telescoping effect [mean (SD)]	Years	5.06 (6.16)	5.88 (5.18)	6.71 (7.52)	0.157

Table 4. Differences in cocaine consumption patterns in patients with CUD according to educational level. % percentage, *CI* confidence interval, *SD* standard deviation, *CUD* cocaine use disorder. *P* value for the Education effect (Kruskal–Wallis H). Significant *P*-value in bold.

cocaine use 7.7 years earlier than women ($U = 31.50$, $p = 0.001$, $\eta 2 = 0.230$) and develop cocaine dependence 6.25 years earlier ($U = 135.50$, $p = 0.003$, $\eta 2 = 0.132$).

Telescoping effect was measured by subtracting the age at onset of cocaine use and the age at development of CUD. However, we did not find significant gender or educational differences.

Gender-based prediction variables in CUD patients. We generated a binary logistic regression model to evaluate the potential of sociodemographic, psychiatric and cocaine related variables as exploratory variables to discriminate between male and female patients. The variables included in the first step were those in which were found gender differences: "educational level" (primary, secondary, university), "psychotropic medication", "anxiolytics", "alcohol use disorder", "cannabis use disorder", "anxiety disorders", "posttraumatic stress disorder", "generalised anxiety disorder", "eating disorders", "bulimia", "type of major depressive disorder" (primary, induced or both), "age at onset of use", and "age at dependence development". Anorexia was removed from the analysis for not having a representative sample for men. All variables met the statistical assumptions of multicollinearity.

Model was prepared using the forward stepwise method and the predictive covariates were restricted to three, which were "alcohol use disorder", "anxiety disorders", and "eating disorders" (Supplementary Table 4S). Hosmer–Lemeshow test indicated good calibration ($\chi^2 = 1.579$, $p = 0.454$) and was able to explain the variation of the dependent variable in 17.3% of the cases according to the Nagelkerke R2 method. It had a classification percentage of 86.8%, showing a high sensitivity for classifying men (100%) and women (12.9%) CUD patients. As seen in Fig. 1, the ROC curve analysis indicated an AUC = 0.712, which represented medium discrimination power. The scatter plot of the predictive probabilities for the patients with CUD indicated that the means were significantly different between both groups ($U = 2870$, $p < 0.001$).

Complex gender-based profile in women attending outpatient treatment for cocaine use. We performed a principal component analysis to evaluate the potential of sociodemographic, psychiatric and cocaine related variables as descriptive variables to explain the different gender profiles of women and men who attend to clinical cocaine treatments. Six components together explained 82.7% of the variance that allowed describing the different profiles of men who attended outpatient treatment for cocaine use (Fig. 2, right). Component 1 explained 18.4% of the total variance and was associated with unemployment (0.923). Component 2 explained 17.2% of the total variance and it was associated with primary education level and had high factor load (0.907). Component 3 explained 14.2% of the total variance and it was related to the single status, with a high factor load (0.857). Component 4 explained 13.1% and was closely associated with divorced, with a high factorial load (0.994). Component 5 explained 10% of the total variance and was related to university studies and

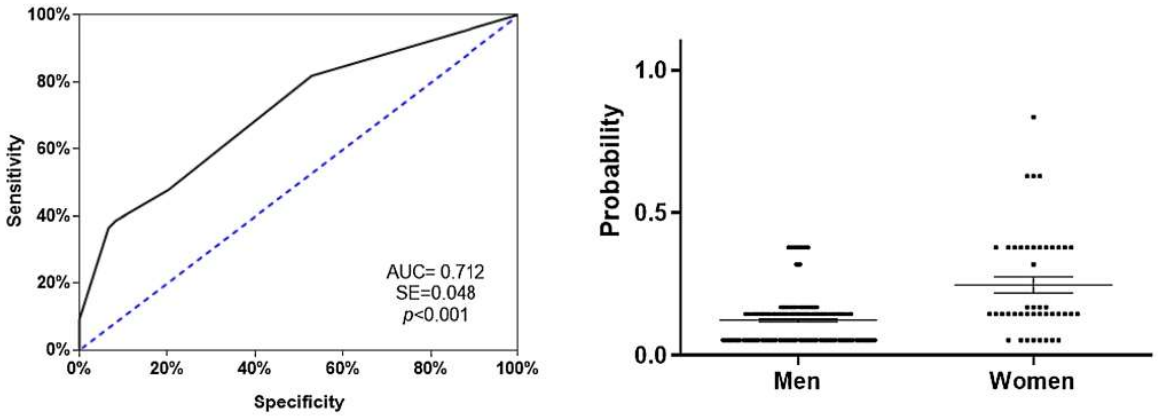

Figure 1. Analysis of the ROC curve (left) using the forward stepwise (conditional) to assess potential of sociodemographic variables and patterns of cocaine use as exploratory variables [area under the curve (AUC = 0.712 (0.048) with $P < 0.001$] to discriminate between men and women patients (N = 204). Dispersion points (right) for the predictive model of logistic regression between men and women. The scatter plot of the predictive probabilities for the patients with CUD indicated that the means were significantly different between both groups (U = 2870. $p < 0.001$).

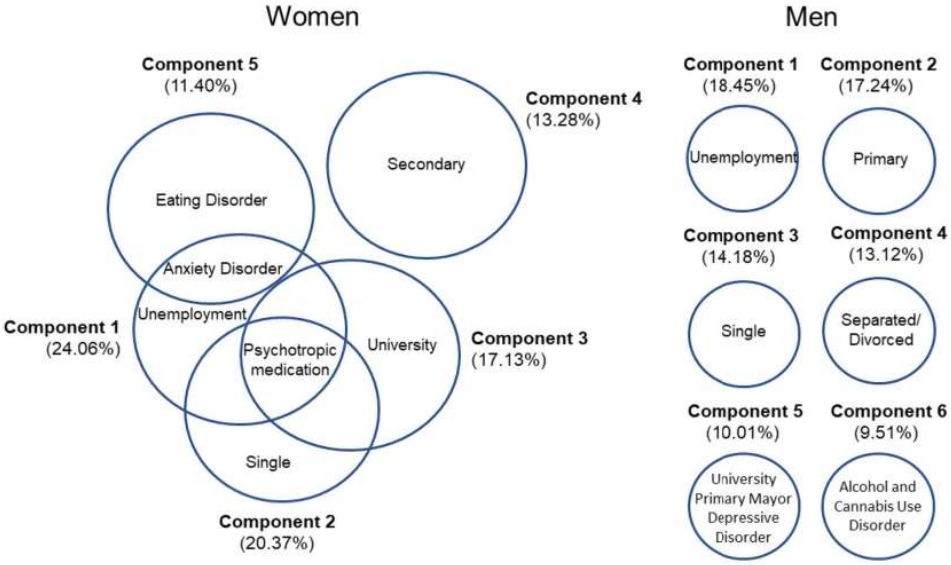

Figure 2. Exploratory principal component analysis for women and men. Differential gender profiles in patients attending outpatient treatment for cocaine use (N = 300). Six components together explained 82.7% of the variance that allowed describing the different profiles of men who attended outpatient treatment for cocaine use (Figure 2, right). Five components together explained 84.5% of the variance that allowed describing the different profiles of women who attended outpatient treatment for cocaine use (Figure 2, left).

primary major depressive disorder, with a high factor load (0.610 and 0.844, respectively). Finally, component 6 explained 9.5%, and was associated with alcohol use disorder and cannabis use disorder, achieving high factor loads (0.761 and 0.728, respectively).

Five components together explained 84.5% of the variance that allowed describing the different profiles of women who attended outpatient treatment for cocaine use (Fig. 2, left). Component 1 explained 24.1% of the total variance. It was associated with unemployment, psychotropic medication, and anxiety disorders, with high factor loads (0.908, 0.637, and 0.322, respectively). Component 2 explained 20.4% of the total variance and was associated with the single status and consumption of psychotropic medication (0.908 and 0.377, respectively). Component 3 explained 17.1% of the total variance and it was associated with the level of secondary education and showed a high factor load (0.883). Component 4 explained 13.3% of the total variance, being associated

with university studies and psychotropic medication, with a high factor load (0.900 and 0.401, respectively). Component 5 explained 11.4% of the total variance and was closely associated with anxiety disorders and eating disorders, exhibiting a high factor load (0.720 and 0.813, respectively).

Discussion

This study adds to the growing literature on gender differences in CUD and incorporate information on sociodemographic, medical, psychopathological and cocaine consumption variables. Moreover, it adds a new point of view that investigates how educational achievements can affect the onset of use, the development of dependence and the duration of cocaine addiction in a different way among men and women. In addition, this study shows a comprehensive analysis of gender profiles based on models from patients attending treatment for cocaine use.

There is high prevalence of psychiatric comorbidities associated with CUD, which may coexist with other substance use disorders or other psychiatric disorders[31–34]. Regarding its association with gender-based differences, in our study the most prevalent disorders in women were anxiety disorders, with generalised anxiety disorder and post-traumatic stress disorder being highly common, as also described in other findings[15,20,32]. In the women population, traumatic experiences such as having suffered child abuse and sexual abuse are factors that have been considered predictive for the development of CUD[32,35]. Thus, these women are more likely to have post-traumatic stress disorder, and more likely to have any anxiety disorders in comparison to men[9]. On the other hand, we observed that the women also showed high prevalence of eating disorders and exhibited more depressive disorders of both, induced and primary nature, throughout their lives in comparison to men, as has also been described in other studies[8,14,20].

Concerning men population, we found a frequency of antisocial personality disorders that were similar to by other authors[15,20]. Similarly, we observed that the coexistence of another substance use disorders throughout life was 64.3%, with alcohol use disorders and cannabis use disorders being more common, as reported by other authors[14,33]. Men are more likely to develop polydrug use of most substances except anxiolytics[3]. There is a biological and social basis for understanding the comorbidity between alcohol use disorder and CUD, since pathological alcohol use enhances cocaine use and vice versa[36]. It is worth noting that the processes of gender violence are more evident in individuals who have concomitant pathological use of these two substances[37].

Thus, if the pathological use of other substances of abuse (especially alcohol), as well as evident lack of effective pharmacological therapies are added to those psychiatric comorbidities, the evolution of these patients consequently worsens, being more vulnerable to relapse and distancing them from primary health resources and deepening their social stigmatisation[38,39].

Related to the variables associated with cocaine consumption, we observed that women developed cocaine dependence two years later than men (28 vs. 26 years), as other authors have also indicated[5,15]. However, we did not find a faster process in women from the first use of cocaine to the development of CUD (telescoping effect), nor did we observe greater severity of addiction in comparison to men, as described in other studies[40,41]. In addition, we did not find gender-based differences in other variables such as the age at onset of use, duration of abstinence, number of abstinences, or the duration of the disorders, as reported by other authors[9,15].

Despite the several gender-based differences related to drug use have been described, healthcare services do not consider the need for a different medical approach for women and men, in spite of demonstrating deserving characteristics of special attention[18]. Our model showed that women and men had different clinical characteristics that should be reflected when designing therapeutic policies. Therefore, it is important to take into account the results of the logistic regression analysis carried out in which appear as predictors the variables: "alcohol use disorder", "anxiety disorders", and "eating disorders". We believe that this class of models—based on sociodemographic variables, patterns of cocaine use, and associated psychiatric comorbidities—could provide information about the aetiology and the progression of the addiction, individually and specifically. They could be useful guides for professionals both at preventive and clinical levels. In this way, preventive measures could be determined and better therapeutic strategies will be developed to improve the quality of treatment.

Furthermore, our results indicated that the profiles of women who sought treatment were different and more complex. The most repeated male profile was that of unemployed men, whereas the most frequent female profile was that of unemployed women who consumed psychotropic medication and had anxiety disorders. This fact means that they had come to the centres in worse psychiatric state than men. In addition, they were at risk of social exclusion and vulnerability. In other words, a triple stigma was observed: being a woman, addiction, and mental disorder.

This characteristic profile in women could indicate a delay in entering the treatment circuits, and we consider that this could be motivated by social, cultural, and medical reasons. Women consume in a less exposed way, since addictions continue to be poorly accepted socially, thus constituting a reason for stigma and implying less family and social support in the female[6,42]. Requests for treatment decreases due to the legal or social repercussions that the consumption of substances could cause to women during the periods of motherhood and education of their children. It is also important to emphasise the pressure exerted by the family members so that the treatments are as brief as possible and the woman can return to perform the household chores, often sacrificing treatment[43]. These aspects explain the low rate of women's admissions to treatments, since they only represented 15% of the total sample of our study, like other studies[20,44].

In addition, the three most frequent profiles of women seeking treatment for cocaine use had the use of psychotropic medication as a common factor, even though two of the profiles did not exhibit any correlation with a specific psychiatric symptom, which could reflect the medical tendency to prescribe this type of psychotropic drugs to women in great proportion[21].

Finally, there are very few studies that relate educational levels to the use of cocaine and much less with its interaction with gender. We observed the protective effect that the educational level exerts on the development

of cocaine addiction: the onset and development of CUD occurred earlier when patients had a lower educational level and these had a longer duration of CUD, whereas patients with university studies started use and developed CUD later. This has also been observed in alcohol, since young people who had dropped out of secondary education or university studies were at higher risk of developing abuse in adult life, compared to those who had completed secondary school or university[45].

The CUD has also been related to executive dysfunctions that compromise efforts to initiate or maintain abstinence and affects the functionality and therapeutic effectiveness of patients[46,47]. Regarding the relationship between gender and educational level, women and men with low educational level started cocaine use much earlier than those with secondary studies. This is particularly important to women with primary education, since they could have a higher risk of gender discrimination and illicit drug use[27]. However, the age at onset of cocaine use and the development of CUD is still earlier in men compared to women who have a low educational attainment.

All this evidence places the educational level as a risk factor or a protective factor depending on the academic achievements, occupying a fundamental role in the appearance and course of addictive disorders. Low educational level can predispose to the emergence of earlier and more severe CUD. While high educational level can prevent or delay the onset of consumption and the subsequent development of CUD, as well as provide better cognitive skills that can promote therapeutic success. Therefore, prolonging the years of compulsory education, as well as implementing cognitive rehabilitation programmes in treatment centres could be extremely useful both for the prevention and for the evolution of patients[48].

In conclusion, women and men have different clinical characteristics that should be considered when designing therapeutic policies for patients with CUD. The educational level plays a fundamental role in the onset and progression of CUD, which is why it is essential to focus attention on it. Therefore, the progressive equality between women and men in the consumption of addictive substances leads us to be even more rigorous in the development of gender-based therapies.

Conclusion remarks

- Women have lower percentages of attendance at treatment centres.
- Women consume more prescribed psychotropic medication, especially benzodiazepines.
- Women have higher prevalence of anxiety disorders and eating disorders, particularly posttraumatic stress disorder, generalised anxiety disorder, anorexia, and bulimia.
- Men have higher rates of other substance use disorder as alcohol and cannabis use disorders.
- Women develop cocaine dependence later than men (28 vs 26 years).
- Patients with primary and secondary educational level start use and develop cocaine dependence early than those with university studies.
- Patients with primary studies suffer more years of CUD than those with secondary educational level.
- Women with primary educational attainment start cocaine use much earlier than those with secondary studies.
- Men with primary educational level start use and develop cocaine dependence early than those with secondary and university studies. Similarly, men with secondary education develop cocaine dependence early than those with university studies.
- Among patients with primary educational level, men start use and develop cocaine dependence much earlier than women.
- Alcohol use disorder, anxiety disorders and eating disorders demonstrated to be good variables to classify between men and women (86.8%).
- The profiles of women who sought treatment for cocaine use were more complex than those of men. They come to the centres in worse psychiatric and social states.

Limitations and prospects

The present study has several limitations that should be considered in further research. First, the small number of female populations was relevant from a clinical perspective, though relatively small from a statistical perspective. Second, these results can only be interpreted in a clinical setting and not in the general population; therefore, caution should be exercised when extrapolating the data to other population settings. Third, we can mention the recall bias of the interviewed patients, due to the use of retrospective diagnostic tools.

In the future, we should consider a series of prospects for improving this type of studies addressing populations with CUD. Firstly, longitudinal studies could be conducted in order to assess the evolution and prognosis of psychiatric comorbidities and severity of the addictions. Also, these studies could assess the cognitive abilities before and after treatments, particularly according to the educational levels of the patients. Secondly, the number of female patients should be increased in the studies. Gender-based comparisons would be much more robust from a statistical point of view. Finally, visibility of this under-diagnosis reality in the population with CUD could be increased with the aim of designing specific therapies according to the characteristics of each gender.

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Author contributions
P.A. and F.R.F., were responsible for the study concept and design. J.J.R., M.F., and M.P., recruited participants. N.R.-O., M.F., A.S.M. and N.G.-M. contributed to the acquisition of psychiatric data by means of interviews. J.S., A.S., F.J.P., and N.R.-O. assisted with data analysis and interpretation of findings. N.R.-O., F.J.P. and P.A. drafted the manuscript. F.R.F., J.S., A.S. and F.J.P. provided critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests
The authors declare no competing interests.

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Conclusiones parciales del Estudio 2:

- Las mujeres tienen menores porcentajes de asistencia a los centros de tratamiento y
- Las mujeres consumen más medicación psicotrópica prescrita, especialmente benzodiazepinas.
- Las mujeres tienen una mayor prevalencia de trastornos de ansiedad y trastornos alimentarios, en particular el trastorno de estrés postraumático, el trastorno de ansiedad generalizada, la anorexia y la bulimia.
- Los hombres tienen tasas más altas de trastornos por consumo de otras sustancias, como trastornos por consumo de alcohol y cannabis.
- Las mujeres desarrollan la dependencia de la cocaína más tarde que los hombres.
- Los pacientes con nivel educativo primario y secundario se inician en el consumo y desarrollan dependencia a la cocaína antes que aquellos con estudios universitarios.
- Los pacientes con estudios primarios sufren más años el trastorno por uso de cocaína que aquellos con nivel educativo de secundaria.
- Las mujeres con nivel educativo primario comienzan a consumir cocaína mucho antes que aquellas con estudios secundarios.
- Los hombres con nivel educativo primario inician el consumo y desarrollan dependencia a la cocaína más temprano que aquellos con estudios secundarios y universitarios. Del mismo modo, los hombres con educación secundaria desarrollan dependencia a la cocaína más temprano que aquellos con estudios universitarios.
- Entre los pacientes con nivel educativo primario, los hombres inician el consumo y desarrollan dependencia a la cocaína mucho antes que las mujeres.
- El trastorno por consumo de alcohol, los trastornos de ansiedad y los trastornos alimentarios demostraron ser buenas variables para clasificar entre hombres y mujeres (86,8%).

- Los perfiles de las mujeres que buscaban tratamiento por consumo de cocaína eran más complejos que los de los hombres. Acuden a los centros en peores estados psiquiátricos y sociales.

4.3. Estudio 3:

Factor de Crecimiento Endotelial Vascular como Potencial Biomarcador de Neuroinflamación y Deterioro Cognitivo Fronal en Pacientes con Trastorno por Uso de Alcohol.

"Vascular Endothelial Growth Factor as a Potential Biomarker of Neuroinflammation and Frontal Cognitive Impairment in Patients with Alcohol Use Disorder"

biomedicines

MDPI

Article

Vascular Endothelial Growth Factor as a Potential Biomarker of Neuroinflammation and Frontal Cognitive Impairment in Patients with Alcohol Use Disorder

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Abstract: (1) Background: Alcohol Use Disorder (AUD) is associated with functional disruption of several brain structures that may trigger cognitive dysfunction. One of the mechanisms of alcohol-associated cognitive impairment has been proposed to arise from its direct impact on the immune system, which culminates in the release of cytokines and chemokines which can eventually reach the brain. Alcohol can also disrupt the blood–brain barrier, facilitating the penetration of pro-inflammatory molecules throughout vascular endothelial growth factor A (VEGFA). Thus, alcohol-induced alterations in chemokines and VEGFA might contribute to the neuroinflammation and cognitive impairment associated with AUD. (2) Methods: The present cross-sectional study investigates whether patients with AUD (n = 86) present cognitive disability associated to alterations in plasma concentration of SDF-1, fractalkine, eotaxin, MCP-1, MIP-1α and VEGFA when compared to control subjects (n = 51). (3) Results: The analysis indicated that SDF-1 and MCP-1 concentrations were higher in AUD patients than in controls. Concentrations of VEGFA were higher in AUD patients with severe frontal deficits, and the score of frontal lobe functions was negatively correlated with VEGFA and fractalkine. Acute alcohol effects on VEGFA plasma levels in healthy volunteers demonstrated the induction of VEGFA release by heavy alcohol drinking. VEGFA was positively correlated with pro-inflammatory chemokines in AUD patients with frontal cognitive impairment. (4) Conclusions: we propose VEGFA/chemokine monitoring as biomarkers of potential cognitive impairment in AUD patients.

Keywords: alcohol use disorders; addiction; VEGFA; blood–brain barrier; chemokines; fractalkine; neuroinflammation; cognitive dysfunction; neurodegeneration; dementia

1. Introduction

Alcohol use disorder (AUD) is one of the main global health problems, carrying a significant social and economic burden. Alcohol abuse is responsible for more than 3 million deaths annually in the world, with the highest rates of alcohol consumption being in the European Union [1]. In Spain, alcohol is the most consumed drug among the general population (15–64 years): 91.2% at some time in their life, 75.2% in the last year, 62.7% in the last 30 days and 7.4% daily during the last month [2].

Among medical consequences related to AUD, we can highlight the induction of liver and pancreatic disease [3,4], psychiatric comorbidities, and other substance use disorders throughout life [5]. Major depressive disorders and anxiety disorders are the most prevalent comorbid psychiatric disorders, whereas cocaine and cannabis misuse are the most frequent comorbid substance use disorders associated with AUD [6,7]. Furthermore, a growing number of studies indicate that alcohol abuse is a major contributor to the development of any type of dementia, especially when there is an early onset of the cognitive impairment [8,9]. In addition, lifetime presence of chronic alcohol dependence has been suggested as an independent risk factor for the development of dementia. Thus, alcohol-related dementia (ARD) has been reported to be one the most prevalent, especially in young men (from 8.27 per 100,000 to 25.6% in several study populations) [10]. Moreover, heavy alcohol consumption has been related with a rapid progression of cognitive decline in aging [11]. According to these data, it is widely known that chronic alcohol consumption has a profound impact on brain structures that support higher cognitive functions [12–14].

Regarding molecular mechanisms mediating alcohol abuse-associated cognitive impairment, it is thought that it is derived from alcohol-induced neuroinflammation mediated by oxidative stress and by the release of proinflammatory signaling molecules (i.e., chemokines/cytokines), ultimately leading to neuronal apoptosis and even necrosis. This process may eventually lead to a permanent derangement of cognition, resulting in dementia. Increasing evidence supports an essential role for Toll-like receptors (TLRs) in alcohol-induced neurodegenerative disease [15]. Recent studies have observed that alcohol stimulates brain immune cells (microglia and astrocytes) by activating TLR (mainly TLR4) and NOD-like receptors. This activation culminates in the production of proinflammatory cytokines and chemokines, leading to neuroinflammation and neuronal damage in the cortex and the hippocampus. Hence, activation of TLR4 by ethanol triggers fast downstream signaling pathways such as mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-kB), eventually promoting the expression and release of cytokines, chemokines, and inflammatory mediators [16,17]. These events can be potentiated by alcohol-induced neuroinflammation inflammatory signals at peripheral tissues (i.e., gut, liver or pancreas) reaching the brain through ethanol's disruption of the blood–brain barrier [18]. Thus, substantial evidence suggests that alcohol impacts the immune system and induces an up-regulation of cytokines and chemokines which are associated with behavioral changes and cognitive impairment [19,20].

Chemokines (chemotactic cytokines) are immune signals involved in cellular migration and intercellular communication. These proteins also act as modulators in neuronal transmission and contribute to communication between glia and neuronal cells [21,22]. In addition, cytokines are involved in the regulation of cell development, survival, and regeneration of the central nervous system [23,24]. These signals are important components of the neuroimmune system that contribute to neuronal activity, neuroendocrine function, brain development, synaptic plasticity, and circuity of mood in drug addiction [25,26]. Furthermore, chemokine decompensation described in plasma, serum and cerebrospinal fluid has been associated with several psychiatric and neurodegenerative diseases such as mild

cognitive impairment, Alzheimer's disease, Parkinson's disease, schizophrenia, bipolar disorder and major depression [27–30]. However, despite the interaction between alcohol and immunological mediators having been well investigated [31,32], little is known about whether these immunoinflammatory signals impact the development of AUD-associated cognitive impairment. Long-lasting brain induction of the proinflammatory cytokines tumor necrosis factor alpha (TNFα), interleukin (IL)-1β and monocyte chemoattractant protein-1 (MCP-1) and the anti-inflammatory cytokine IL-10 have been related to microglial activation and reduced neurogenesis in mice exposed to LPS endotoxin after ethanol treatment [33]. However, plasma chemokines have been evaluated almost exclusively in the context of liver disease in alcohol-dependent patients [34,35].

On the other hand, Vascular Endothelial Growth Factor A (VEGFA) could be a potential candidate for explaining how alcohol facilitates both infiltration and inflammation in the brain. VEGFA is a protein that belongs to the family of growth factors and is commonly known for its role in angiogenesis and vascular permeability. In addition, VEGFA plays a fundamental role in the development and adult nervous system since it is involved in the extension and complexity of the microvasculature that supplies the necessary nutrients and oxygen in the brain. In this way, the effects of VEGFA on the nervous system have been related to neuroprotection, neurogenesis, and synaptic plasticity through the stimulation of neural stem cells and safeguarding the integrity of the blood–brain barrier [36]. Therefore, changes in VEGFA concentrations could affect the function and survival of neurons by not providing enough nutrients or producing hypoxia, which has been related to the deterioration of cognitive function [37]. Furthermore, VEGFA is a potent vasodilator and angiogenic factor released under hypoxic and stressful conditions via endothelial nitric oxide synthase [38,39]. Altered levels of VEGFA have been related to several neurodegenerative and neurological disorders, such as Alzheimer's disease, vascular dementia and stroke [36]. Nevertheless, VEGFA is still poorly understood in the field of substance use disorders. Heberlein (2010) observed that VEGFA serum levels increase during alcohol withdrawal, and it might be intimately associated with alcohol intoxication and the severity of the addiction reflected by recurrent episodes of alcohol intoxication [40]. In addition, augmented VEGFA levels have been found in alcoholic liver disease patients compared to controls, showing a positive association with cholestatic enzymes [41].

Considering the previous antecedents, in the present study, we investigated the potential association of plasma concentrations of the chemokines stromal cell-derived factor 1 (SDF-1), fractalkine, eotaxin, MCP-1, macrophage inflammatory protein 1 alpha (MIP-1α) and the trophic factor VEGFA to frontal cognitive impairment in AUD patients. In addition, to fully understand the effects of alcohol on VEGFA, we studied the plasma concentration of this trophic factor after acute alcohol intake. The ultimate goal was to identify a potential link between AUD-associated cognitive impairment and plasma levels of VEGFA and chemokines that might be eventually useful for clinical purposes.

2. Materials and Methods

2.1. Recruitment and Screening of Participants

The cross-sectional study included 137 Caucasian volunteers divided into two groups: 86 abstinent AUD patients (alcohol group) in outpatient treatment and 51 control subjects (control group) matched by age, body mass index (BMI) and proportion of sex. Patients were recruited at the Psychiatry Service of the Hospital Universitario 12 de Octubre (Madrid, Spain) and Centro Provincial de Drogodependencias (Málaga, Spain). Control participants were included from databases of healthy subjects (without presence of cognitive impairment, medical diseases and substance use disorders) of the Biobanco Nacional de DNA. In addition, we performed a brief frontal neuropsychological evaluation in 59% of AUD patients (Figure 1).

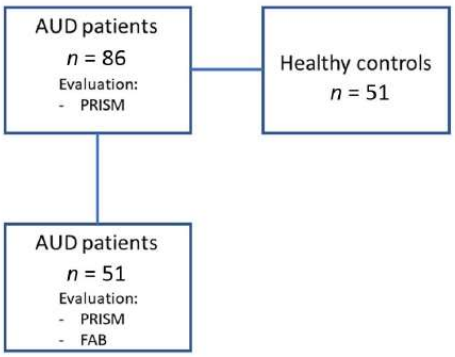

Figure 1. Diagram showing the design of the cross-sectional study.

AUD Patients and Control Volunteers

To be included in the present study, participants had to meet the following inclusion criteria: people aged 18 to 65 years in the abstinence phase, being in outpatient treatment and willingness to participate by signing the informed consent. As we wanted to control for potential interferences in plasma concentrations of chemokines and VEGFA, the exclusion criteria included: use of anti-inflammatory drugs or MAOI's, personal history of long-term inflammatory disease or cancer, pregnant or breast-feeding women and infectious diseases such as Hepatitis C, Hepatitis B and HIV.

An additional group of healthy subjects was recruited at Hospital German Trias I Pujol from Badalona, Spain, to investigate the acute actions of alcohol on VEGFA plasma concentrations. The study design was simple blind, non-randomized, non-controlled study of the experimental administration of alcohol simulating a "binge drinking" episode. Ten healthy male subjects were recruited and administrated an alcoholic beverage containing 100 g of alcohol (312 mL vodka Absolut®, Ahus, Sweden) were mixed with 588 mL of orange soda [Trina® Orange No gas. Suntury Limited, Dōjimahama, Japan], total volume 900 mL. The alcoholic beverage was distributed in 6 identical glasses (volume 150 mL) and consumed continuously over a 2 h period (15 min per glass). Participants were selected after a general medical examination to exclude any psychopathological condition. Subjects signed an informed consent prior to participation and were economically compensated for any inconvenience caused during the trial. The participants had a mean age of 22 ± 2 years, mean weight 73.0 ± 9.2 kg, mean height 180 ± 6.5 cm and index body mass (IBM) 22.5 ± 1.9 kg/m^2. They drank an average of 13.7 ± 8.3 g of alcohol per day and reported a mean 1.3 ± 1.7 alcohol binge episodes per month.

2.2. Ethical Statement

Written informed consent was obtained from each participant after a complete description of the study. All participants had the opportunity to discuss any questions or problems. For the cross-sectional study, the design and the recruitment protocols were approved by the Ethics Committee of the Hospital Regional Universitario de Málaga (PND 2019/040). The acute alcohol administration experiment protocol was approved by the local Human Research Ethics Committee (CEI Hospital Universitari Germans Trias i Pujol, Badalona, Spain) and registered at ClinicalTrials.gov (NCT02232789). All procedures were in strict accordance with the Ethical Principles for Medical Research with Human Subjects adopted in the Declaration of Helsinki by the World Medical Association (64th General Assembly of the WMA, Fortaleza, Brazil, October 2013) and Recommendation No. R (97) 5 of the Committee of Ministers to the Member States on the protection of medical data (1997), and the Spanish law on data protection [Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and the free circulation of such data, and repealing Directive

95/46/EC (General Data Protection Regulation)]. All collected data received code numbers to maintain privacy and confidentiality.

2.3. Psychiatric and Neuropsychological Evaluation

The Spanish version of the PRISM (Psychiatric Research Interview for Substance and Mental Diseases) diagnostic interview was used for the evaluation of substance use disorders and other psychiatric disorders according to the criteria of the DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, 4th edition). The PRISM is a semi-structured interview with good psychometric properties in the evaluation of substance use disorders and in the main comorbid psychiatric disorders related to the substance use population [42,43].

The neuropsychological evaluation was performed using the Spanish version of the Frontal Assessment Battery (FAB) for the diagnosis related to frontal lobe dysfunctions [44] that have demonstrated reliability and good psychometric properties. The total FAB score was obtained from 0 to 18 evaluating the subdomains respectively: grasp, go-no-go, conflictive, lexical fluency, and motor skills. A cut-off point lower than 16 separates normal frontal deficits from mild ones, and a cut-off point lower than 13 separates mild and severe frontal syndrome.

2.4. Obtaining Plasma Samples

Blood samples were obtained in the morning after fasting for 8–12 h (before psychiatric interviews). Venous blood was extracted into 10 mL K2 EDTA tubes (BD, Franklin Lakes, NJ, USA) and immediately processed to obtain plasma. Blood samples are centrifuged at 2200 g for 15 min (4 °C) and individually tested for infectious diseases using 3 commercial rapid tests for HIV, Hepatitis B and Hepatitis C (Strasbourg, Cedex, France). Finally, the plasma samples were individually aliquoted, recorded and stored at −80 °C until further analysis.

2.5. Multiplexed Bead Immunoassay

Plasma concentrations of SDF-1, eotaxin, MIP-1, MCP-1, fractalkine and VEGFA were measured by using a human custom 7-ProcartaPlex bead immunoassay kit (Invitrogen, cat. no. PPX-07-MXH6ANW, Waltham, MA, USA) in a Luminex xMAP®® technology—MAGPIG system (ThermoFisher, Waltham, MA, USA). Sensitivity was approximately 13, 33, 12, 51, 39 and 78 pg/mL for SDF-1, eotaxin, MIP-1α, MCP-1, fractalkine and VEGFA, respectively. Mean intra-assay variation (%CV replicates) was 5.3, 9.3, 10.3, 7.1, 11.1 and 12.1%, respectively, and mean inter-assay variation (%CV) was 29.7, 30.1, 44.6, 48.5, 36.7 and 19.9%, respectively, for all analyses. The minimum detectable concentration values were attributed to missing values that were under the standard curve.

2.6. Statistical Analysis

All data in tables are expressed as numbers and percentage of subjects [*n* (%)] or means and standard deviations (SD). The significance of the differences in the qualitative variables was determined through Fisher's exact test (Chi-square). The normal distribution of the variables was assessed using Lilliefors corrected Kolmogorov-Smirnov test. For continuous variables that did not meet the assumption of normality, statistical analyses were performed using non-parametric Mann–Whitney *U*-test for comparisons between two groups and Spearman for correlations. For continuous variables that met the assumption of normality, we used an ANOVA with repeated measures. Lastly, a principal components analysis with varimax rotation and bivariate relationships (correlation) was performed to determine the different profiles of alcohol-abstinent patients with cognitive decline. Only variables with a factor load of at least 0.3 (i.e., those that share at least 10% of the variance with a factor) were used for the interpretation. A *p*-value less than 0.05 was considered statistically significant. Statistical analyses were carried out using GraphPad Prism version 5.04 and IBM SPSS Statistical version 22 (IBM, Armonk, NY, USA). For the time-course analysis of the acute effects of alcohol on VEGFA, ANOVA with repeated measures design was selected. In

the case of plasma concentrations of VEGFA a non-parametric Friedman test for repeated measures was selected. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Sociodemographic Characteristics

Table 1 shows a socio-demographic description of the total sample. We selected 86 abstinent patients with AUD diagnosis and 51 healthy control subjects matched for sex, age and BMI. The mean age of the AUD group was 44 years and the 81% were men with a BMI index of 26. A significant difference was observed between the two sample groups when educational level and occupation was analyzed ($p < 0.022$, $p < 0.001$).

Table 1. Socio-demographic characteristics of the total sample.

		Total Sample (n = 141)			
Variables		**Control Group (n = 52)**	**Alcohol Group (n = 89)**	**Statistic**	***p*-Value**
Age (Mean ± SD)	Years	47.14 ± 5.29	44.16 ± 11.88	1867.50 [1]	0.056
Body Mass Index (Mean ± SD)	Kg/m^2	27.15 ± 3.59	26.36 ± 4.84	1907.50 [1]	0.117
Sex [n (%)]	Women Men	17 (32.70) 35 (67.30)	17 (19.10) 72 (80.90)	3.313 [2]	0.069
Education Degree [n (%)]	Elementary Secondary University	13 (25) 20 (38.50) 19 (36.50)	36 (40.40) 38 (42.70) 15 (16.90)	7.672 [2]	**0.022**
Occupation [n (%)]	Employed Unemployed Retired Other	45 (86.50) 0 3 (5.80) 4 (7.70)	19 (21.30) 39 (43.80) 13 (14.60) 18 (20.20)	59.081 [2]	**<0.001**

[1] Value was calculated with Mann–Whitney *U*-test. [2] Value was calculated with Fischer's exact test. Bold values are statistically significant for $p < 0.05$.

3.2. Alcohol-Related Variables in AUD Group

The variables related to the AUD group were evaluated and are described in Table 2. The mean age at first drink of alcohol was 15 years, while the average age of the AUD onset was 26 years with 15 years of problematic alcohol use. The mean of severity criteria of addiction was 8 (based on DSM-5) and they had a length of 322 days of abstinence at the moment of the evaluation.

Table 2. Clinical characteristics of AUD patients with and without frontal cognitive impairment.

		AUD Group				
Variables		**Total AUD (n = 89)**	**AUD with FCI (n = 28)**	**AUD without FCI (n = 23)**	**Statistic**	***p*-Value**
Age at first alcohol use (Mean ± SD)	Years	14.69 ± 4.027	14.42 ± 3.62	15.62 ± 3.71	271 [1]	0.330
Age at onset of AUD (Mean ± SD)	Years	25.99 ± 9.591	28.38 ± 12.31	26.20 ± 9.58	266.50 [1]	0.417
Length of AUD diagnosis (Mean ± SD)	Years	15.06 ± 11.314	11.46 ± 8.96	15.19 ± 11.40	228 [1]	0.334
Severity criteria (Mean ± SD)	Criteria [1–11]	8.09 ± 2.114	7.96 ± 2.20	8.52 ± 2.32	299.50 [1]	0.666
Length of abstinence (Mean ± SD)	Days	322.12 ± 908.545	63.46 ± 60.69	432.95 ± 1069.93	305.50 [1]	0.961

Table 2. *Cont.*

Variables		AUD Group				
		Total AUD (*n* = 89)	**AUD with FCI (*n* = 28)**	**AUD without FCI (*n* = 23)**	**Statistic**	***p*-Value**
Comorbid substance use disorders [*n* (%)]	Tobacco	69 (77.50)	21 (75)	21 (91.30)	2.264 [2]	0.132
	Cocaine	43 (48.30)	12 (42.90)	11 (47.80)	0.126	0.723
	Cannabis	19 (21.30)	4 (14.30)	4 (17.40)	0.090	0.764
	Sedatives	7 (7.90)	-	4 (17.40)	5.180	0.023
Comorbid psychiatric disorders [*n* (%)]	Mood	44 (49.4)	14 (50)	8 (34.80)	1.192 [2]	0.275
	Anxiety	24 (27)	6 (21.40)	5 (21.70)	0.001	0.979
	ADHD	19 (21.30)	3 (10.70)	2 (8.70)	0.057	0.811
	Personality	14 (15.70)	5 (17.90)	4 (17.40)	0.002	0.966
	Psychotic	8 (9)	3 (10.70)	1 (4.30)	0.694	0.405
Psychiatric medication [*n* (%)]	Antidepressants	46 (51.70)	17 (60.70)	12 (52.20)	0.375 [2]	0.540
	Anxiolytics	56 (62.90)	15 (53.60)	18 (78.30)	3.370	0.066
	Antipsychotics	10 (11.20)	2 (7.10)	1 (4.30)	0.175	0.676
	Disulfiram	35 (39.30)	14 (50)	10 (43.50)	0.216	0.642
	Anticraving	9 (10.10)	5 (17.90)	1 (4.30)	2.117	0.204

Abbreviations: FCI = Frontal Cognitive Impairment, ADHD = attention deficit hyperactivity disorder (childhood). [1] Value was calculated with Mann–Whitney *U*-test. [2] Value was calculated with Fischer's exact test. Bold values are statistically significant for $p < 0.05$.

Regarding other substance use disorders, tobacco (77%) and cocaine (48%) were the most prevalent drugs among AUD patients. In addition, an elevated prevalence of other comorbid psychiatric disorders was observed, with lifetime mood and anxiety disorders being the most frequently diagnosed, in 49% and 27%, respectively. Furthermore, 87% of the abstinent alcohol patients received psychiatric medication during the last year: anxiolytics (63%), antidepressants (52%), antipsychotics (11%) and anticraving (10%). Finally, 39% of the AUD group was treated with disulfiram.

The neuropsychological evaluation revealed that 55% of the AUD group showed some deficits related to frontal cognition (assessed by FAB): 45% did not have frontal deficits, 31% had mild cognitive deficits, and 23% showed severe cognitive impairment. We observed a high prevalence of sedative use disorders in patients without frontal cognitive impaired compared to patients with cognitive impairment ($U = 5.180$, $p = 0.023$). However, we did not find significant differences in other alcohol-related variables between patients with and without frontal cognitive impairment.

3.3. Plasma Concentrations of VEGFA and Chemokines in Abstinent AUD Patients

The impact of alcohol dependence on plasma concentrations of VEGFA and chemokines was studied in the total sample using Mann–Whitney *U*-test. Plasma concentrations of SDF-1 ($U = 1615$, $p = 0.010$) and MCP-1 ($U = 1354$, $p < 0.001$) were significantly higher in the alcohol group compared to the control group (Figure 2). However, we did not observe major differences in MIP-1α, eotaxine, fractalkine and VEGFA between the alcohol group and the control group (Table S1).

Moreover, correlation analysis between plasma concentrations of VEGFA and chemokines and age at first alcohol use, age at onset of AUD, length of AUD diagnosis, severity criteria, and length of abstinence were conducted in these AUD patients. Plasma levels of SDF-1, eotaxin and VEGFA were found to be significantly correlated with alcohol addiction severity based on alcohol criteria (rho = 0.211, $p < 0.048$; rho = 0.250, $p < 0.018$; rho = 0.234, $p < 0.027$, respectively) (Table S2).

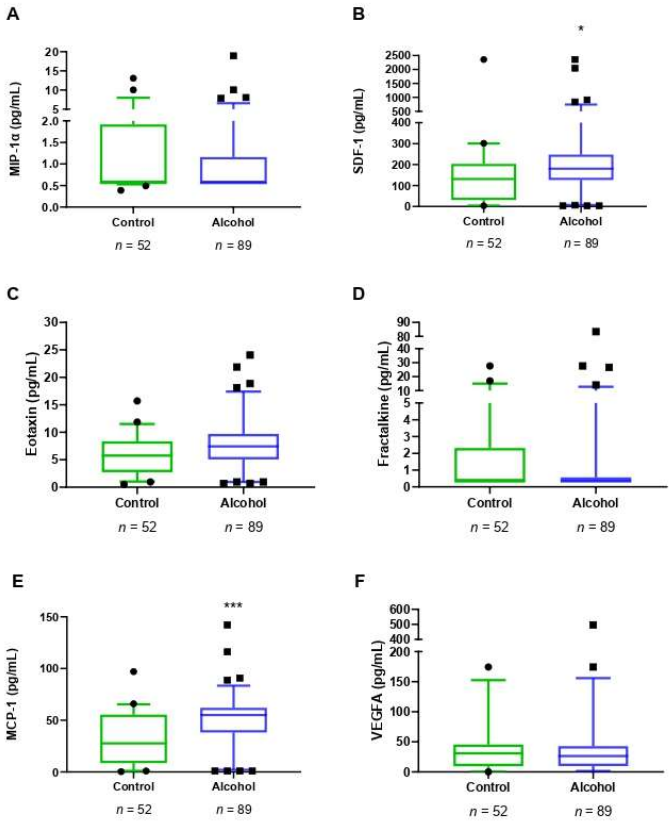

Figure 2. Plasma concentrations of VEGFA and chemokines in the alcohol group vs the control group (n = 141). (**A**) MIP-1α (pg/mL), (**B**) SDF-1 (pg/mL), (**C**) Eotaxin (pg/mL), (**D**) Fractalkine (pg/mL), (**E**) MCP-1 (pg/mL), and (**F**) VEGFA (pg/mL). Box and whiskers plotted at the 5–95 percentile. Dots are individual values. Data were analyzed by Mann–Whitney U-test. (*) $p < 0.05$ and (***) $p < 0.001$ denote significant differences compared with the control group.

3.4. Plasma Concentrations of VEGFA and Chemokines in Abstinent AUD Patients with Frontal Cognitive Impairment

To explore the influence of frontal cognition integrity on plasma concentrations of VEGFA and chemokines, we performed a Kruskal Wallis test with "frontal cognitive impairment" (no, mild, and severe) as a factor. We did not find a significant effect of "frontal cognitive impairment" on plasma concentrations of VEGFA and chemokines (Table S3). However, as shown in Figure 3, circulant levels of VEGFA almost reached the significance (K = 5.404, $p = 0.067$), having higher plasma concentrations of VEGFA the AUD patients with severe frontal cognitive impairment than those without frontal deficits ($U = 72.50$, $p = 0.021$).

3.5. Correlation Analyses between Frontal Cognition and Plasma Concentrations of VEGFA and Chemokines in AUD Patients

For deeper analysis, we explored the relationship between frontal lobe functions (evaluated by FAB) and plasma concentrations of VEGFA and chemokines using Spearman correlations (rho). As shown in Table 3, there was a significant and negative correlation between FAB score and plasma concentrations of VEGFA (rho = −0.290, $p = 0.039$). We also observed a significant and negative correlation between FAB score and plasma concentrations of fractalkine (rho= −0.336, $p = 0.016$). However, we did not find significant

correlations between FAB scores and the plasma concentrations of SDF-1, eotaxin, MIP-1α and MCP-1.

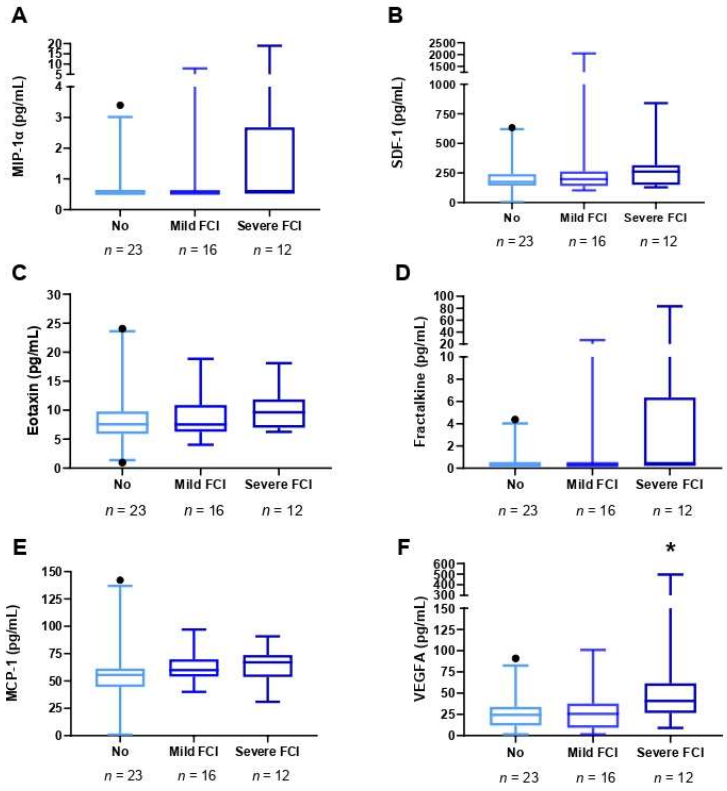

Figure 3. Plasma concentrations of VEGFA and chemokines in AUD patients with frontal cognitive impairment (n = 28). (**A**) MIP-1α (pg/mL), (**B**) SDF-1 (pg/mL), (**C**) Eo-taxin (pg/mL), (**D**) Fractalkine (pg/mL), (**E**) MCP-1 (pg/mL), and (**F**) VEGFA (pg/mL). Box and whiskers plotted at the 5–95 percentile. Dots are individual values. Data were analyzed by Kruskal Wallis test. (*) $p < 0.05$ denote a significant difference compared with AUD patients without frontal deficits. Abbreviations: FCI = Frontal Cognitive Impairment.

Table 3. Correlation analysis between FAB scores and plasma concentrations of chemokines and VEGFA in AUD patients (n = 51). (rho) Spearman's correlation coefficient. Bold values are statistically significant for $p < 0.05$.

Variables	FAB (Score)	
	Rho	*p*-Value
SDF-1 (pg/mL)	−0.228	0.111
Eotaxin (pg/mL)	−0.147	0.303
MIP-1α (pg/mL)	−0.154	0.280
MCP-1 (pg/mL)	−0.203	0.152
Fractalkine (pg/mL)	−0.336	**0.016**
VEGFA (pg/mL)	−0.290	**0.039**

3.6. Correlation Analyses between Plasma Concentrations of VEGFA and Chemokines in AUD Patients with and without Mild Cognitive Impairment

Moreover, we wanted to explore the relationship between plasma concentrations of chemokines and VEGFA depending on the integrity of frontal lobe function (evaluated by FAB) using Spearman correlations (rho). As shown in Figure 4, AUD patients with cognitive impairment displayed strong positive and significant correlations between VEGFA with all chemokines [SDF-1 (rho = 0.787, $p < 0.001$), eotaxin (rho = 0.678, $p < 0.001$), MIP-1α (rho = 0.592, $p = 0.001$), MCP-1 (rho = 0.601, $p = 0.001$), fractalkine (rho = 0.706, $p < 0.001$)], while we only observed a positive and significant correlation between VEGFA and SFD-1 (rho = 0.532, $p = 0.009$) for AUD patients without cognitive impairment (Table S4).

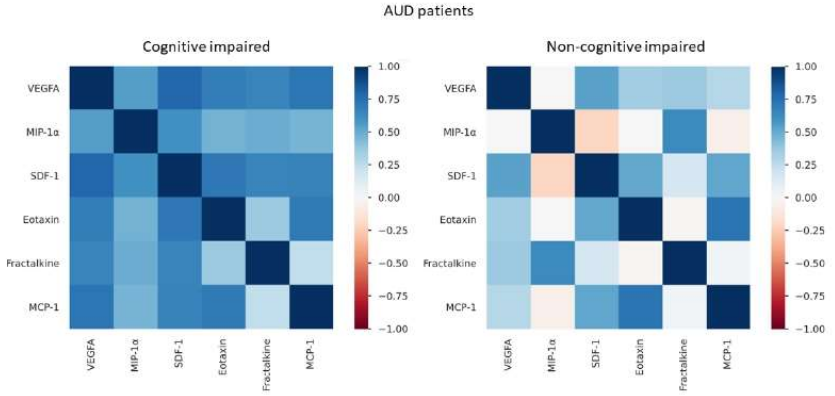

Figure 4. Correlations analysis between VEGFA and chemokines in AUD patients with and without frontal cognitive impairment ($n = 51$). Colors show Spearman's rho correlation coefficient.

3.7. Differential Profiles Associated with VEGFA and Chemokines in AUD Patients with Frontal Cognitive Impairment

To understand the contribution of VEGFA and chemokines in the frontal cognitive decline of AUD patients, a principal component analysis was performed. Two components together explained 80.67% of the variance associated with cognitive impairment in AUD patients (Figure 5). Component 1 explained 56.61% of the total variance and was composed of SDF-1, fractalkine and VEGFA, which had high factor loads (0.984, 0.983, 0.757, respectively). Component 2 explained 24.06% of the total variance and was composed of MIP-1α, eotaxin, MCP-1 and VEGFA, which had high factor loads (0.669, 0.904, 0.807, 0.582, respectively).

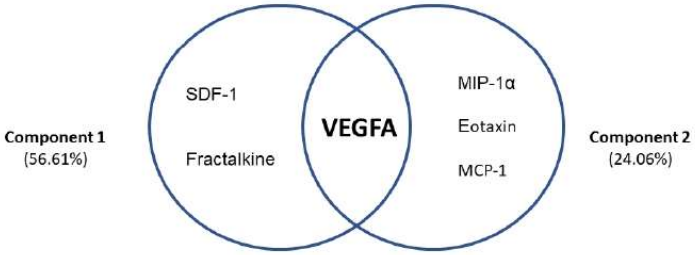

Figure 5. Exploratory principal component analysis in AUD patients with frontal deficits ($n = 51$). Two components together explained 80.67% of the variance associated with frontal cognitive impairment in AUD patients.

3.8. Plasma Concentrations of VEGFA and Chemokines in Comorbid Medical, Psychiatric Medication and Substance Use Problems in AUD Patients

We wanted to investigate whether medical and psychiatric comorbidity, other concomitant substance use disorder or using psychotropic medication could affect plasma concentrations of VEGFA and chemokines in the alcohol group. Plasma concentrations of MIP-1α ($U = 567$, $p = 0.002$) and VEGFA ($U = 552$, $p = 0.005$) were significantly higher in AUD patients with comorbid psychiatric disorder compared to those without psychiatric comorbidity (Table 4). Moreover, plasma concentrations of MIP-1α ($U = 633$, $p = 0.005$) were significantly higher in AUD patients with comorbid substance use disorder compared to those without comorbid substance use disorder (Table S5). Regarding comorbid medical problems and the use of psychotropic medication, we did not observe major differences in plasma levels of chemokines and VEGFA (Tables S6 and S7).

Table 4. Plasma concentrations of chemokines and VEGFA grouped according to comorbid psychiatric disorder. Bold values are statistically significant for $p < 0.05$.

AUD Group ($n = 89$)				
Variables	**Comorbid Psychiatric Disorder ($n = 60$)**	**No Comorbid Psychiatric Disorder ($n = 29$)**	**Statistics**	
	Mean [95% CI]	**Mean [95% CI]**	***U*-Value**	***p*-Value**
SDF-1 (pg/mL)	271.9046 [165.9141–377.8952]	189.3975 [143.9530–234.8421]	831	0.828
Eotaxin (pg/mL)	7.77278 [6.57250–8.97306]	7.47531 [5.57394–9.37668]	806	0.575
MIP-1α (pg/mL)	1.8189 [1.0330–2.6048]	0.6189 [0.5586–0.6793]	567	**0.002**
MCP-1 (pg/mL)	48.9765 [42.4613–55.4917]	48.4279 [36.5812–60.2747]	813	0.618
Fractalkine (pg/mL)	2.0546 [0.6521–3.4570]	0.7399 [0.1778–1.3020]	741	0.160
VEGFA (pg/mL)	36.4530 [28.3181–44.5879]	20.1710 [14.3319–26.0100]	552	**0.005**

3.9. Time Course of Plasma Concentrations of VEGFA after an Acute Administration of Alcohol (100 g) in Healthy Male Volunteers

To clarify how an acute intoxicating dose of alcohol affects circulating levels of VEGFA, we administered 100 g of alcohol to male healthy volunteers whose daily average alcohol intake was of 13.7 ± 8.3. Plasma samples were taken previous to (time 0) and 2, 8, and 24 h after oral ingestion of alcohol. As expected, plasma ethanol level peaked at 2 h after ingestion and decreased to a third of the 2 h concentration 8 h after the ingestion, being undetectable 24 h after oral intake (Figure 6A). Levels of VEGFA were very variable in between these subjects so a non-parametric statistics with repeated measures approach was taken. Data indicated that alcohol modified plasma VEGFA, being elevated 8 h (Figure 6B) after the ingestion of alcohol (Friedman's statistic for $n = 9$, four groups, was 9.26, $p < 0.03$). Analysis of the percentage of change from basal values indicated the alcohol induced a 2-fold change on VEGFA circulating concentrations with respect to the basal values (Figure 6C, ANOVA repeated measures $F(8,24) = 5.14$, $p < 0.001$). Twenty-four hours after the intake of alcohol, the % of change had a non-significative average fold change of 1.7, suggesting that alcohol induced sustained increases of VEGFA. However, this assumption needs to be conclusively determined.

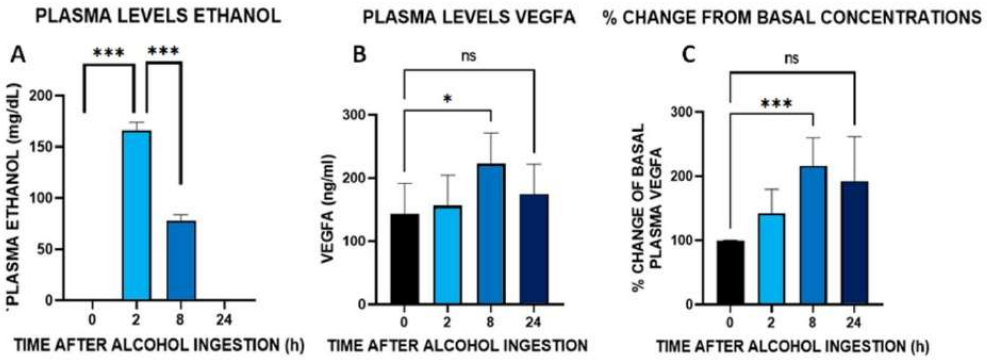

Figure 6. Plasma concentrations of alcohol (**A**) and VEGFA (**B**) at 2, 8 and 24 h after alcohol (100 g) ingestion in male (n = 9–10) healthy volunteers. 0 represents the time of ingestion. Alcohol resulted in increase in plasma concentrations of VEGF 8 h after its ingestion (Friedman's non-parametric test for repeated measures, * $p < 0.05$ versus 0 time). (**C**) Percentage of change of VGEFA calculated over basal concentrations (ANOVA with repeated measures, *** $p < 0.001$ versus 0 time). ns = non-significant.

4. Discussion

While the association between immune signaling and neuropsychiatric disorders has been widely investigated, its link with AUD-related cognitive impairment remains poorly investigated. We lack relevant information concerning how immune mediator-induced proinflammatory states in the brain influence the neuroadaptations derived from chronic alcohol consumption, and how they impact executive functions. In the present study, we unveil a link between plasma concentrations of VEGFA and chemokines with frontal lobe dysfunction in abstinent AUD patients treated in an outpatient setting. The relevance of the present study rests on the confirmation of the link between alcohol-induced dysfunctions in modulators of the blood–brain barrier (i.e., VEGFA) and neuroinflammation (i.e., chemokines) with the presence of cognitive impairment. The main findings are as follows: (i) AUD patients had increased plasma concentrations of SDF-1 and MCP-1 compared to control subjects; (ii) there were higher circulant levels of VEGFA in AUD patients with severe frontal deficits than in those without cognitive impairment; (iii) acute administration of a heavy dose of VEGFA resulted in a delayed increase (8 h after alcohol ingestion) of plasma VEGFA concentration; (iv) the integrity of frontal lobe functions was negatively correlated with VEGFA and fractalkine; (v) plasma concentrations of VEGFA were strongly and positively correlated with all chemokines in AUD patients with frontal deficits but not in those without frontal impairment; (vi) two components together explained 80.67% of the variance associated with frontal deficits in AUD patients, with VEGFA acting as a link between all chemokines; and (vii) plasma concentrations of some chemokines changed in AUD patients with comorbid psychiatric (MIP-1α, VEGFA) and substance use (MIP-1 α) disorders.

A growing literature indicates that the pharmacodynamic action of several drugs involves changes in the neuroimmune signal [45]. Some studies have reported that alcohol-related behaviors are interrupted when the innate immune network is disturbed [46,47]. Thus, Blednov et al. (2005) showed that gene deletion of CCR2, MCP-1 or MIP-1α reduced motivational effects of alcohol consumption in mice [48]. Moreover, Steinar et al. (2019) found an increase in the plasma concentrations of IL-6, IFNγ and MCP-1 in patients with a history of chronic alcohol overconsumption [49]. In agreement with these reports, the AUD patients of the present study displayed higher plasma concentrations of SDF-1 and MCP-1 than control subjects. SDF-1 was also associated (along with eotaxin) with worse severity of alcohol addiction. Even post mortem analysis reported increased expression of MCP-1 in multiple limbic brain regions in alcoholic subjects [50]. Taking into account the high prevalence of cocaine use disorder in AUD patients, it is important to note that extensive preclinical research has reported how the chemokines SDF-1 and MCP-1 pro-

mote cocaine-related behaviors in a brain region specific manner [51–53]. Our group has described that severity of cocaine consumption is related to IL-1β, SDF-1 and fractalkine in cocaine use disorder patients [54]. Furthermore, we found higher plasma concentrations of fractalkine in abstinent cocaine patients with comorbid major depressive disorder than in those without this psychiatric condition [55]. In addition, we demonstrated the induction of a potent fractalkine signaling associated with cocaine-induced sensitization and extinction in mice [56].

Regarding cognitive function, 55% of the AUD patients recruited in the present study displayed some kind of frontal cognitive impairment. Accordingly, executive functions are particularly affected in AUD patients who show deficits in domains related to cognitive control, flexibility, inhibition, planning and working memory [57,58]. However, there are other neuropsychological processes that could be disrupted, including memory, emotion, and social cognition [59]. It is important to note that despite there being evidence of partial recovery of certain cognitive functions after cessation of alcohol intake [13,60], deficits in other domains may remain stable during sobriety [57,61]. Additionally, cognitive impairment can compromise efforts to initiate and maintain abstinence by impacting treatment effectiveness [62]. It is thought that increased vulnerability to alcohol-induced working memory impairment may impact in the ability to moderate alcohol consumption [63] and cognitive training could reduce the number of beverages in these patients [64]. Furthermore, our results indicate that AUD patients have low educational and occupational attainment, which has been related to worse neuropsychological performance, more indicators of neurocognitive disorders, early drug use onset and development of addiction, high-severity substance-related problems, and worse treatment outcomes [65–67]. Thus, excessive alcohol consumption has been linked to worse cognitive functioning in patients with low socioeconomic status operated by educational and occupational achievements [68]. Moreover, a longitudinal study has suggested that an educational level lower than high school and a low job occupation is associated with an increased risk of dementia in alcohol patients [69]. Similarly, our group has recently found that a high educational level could play a protective role in the onset, development, and progression of cocaine use disorders and could also protect against cognitive impairment caused by alcohol consumption throughout life [70,71]. Interestingly, in this study we found a negative association between the state of frontal lobe functions with two signaling molecules, VEGFA and fractalkine, involved in vascular function and neural plasticity.

VEGFA has been related to neuroprotection, neurogenesis, and synaptic plasticity mechanisms in the central nervous system through stimulation of neuronal stem cells and safeguarding the integrity of the blood–brain barrier [36]. Despite VEGFA levels having been linked to several neurodegenerative and neurological disorders [36], their effects could be time dependent. Augmented VEGFA levels in cerebrospinal fluid and plasma have been reported in Alzheimer's disease and vascular dementia patients [72,73] probably as a consequence of hypoperfusion and hypoxia. Nevertheless, improvement in learning and memory after a bilateral carotid artery occlusion has been associated with an increase in VEGFA expression in the hippocampus, which suggests that VEGFA signaling could compensate for cognitive impairment [74,75]. Similarly, partial increases in VEGFA stimulate vasodilation, angiogenesis and neuroprotection mechanisms, which are beneficial for the brain in later stages after cerebral ischemia [76]. However, early VEGFA increases may lead to undesirable effects in cerebral ischemia, such as an increase in blood–brain barrier permeability and infiltration of immune cells inducing neuroinflammation and edema [77–79].

With reference to the latter, in the present study we observed that increases in VEGFA were associated with worse severity of alcohol addiction, severe frontal deficits and the elevation of all chemokines in frontal cognitive impaired AUD patients. Interestingly, in a pilot study with healthy male volunteers with a history of moderate alcohol consumption, we found that acute alcohol administration resulted in a delayed increase in plasma VEGFA, observed 8 h after alcohol intake. Because of the increased vascular permeability induced

by VEGFA, it is reasonable to think that this action of alcohol might facilitate neuroinflammation by opening the blood–brain barrier to pro-inflammatory signals originating in peripheral tissues, especially in the intestine. Supporting this hypothesis, in our principal component analysis, we found that the interaction of chemokines and VEGFA explained the 80.67% of the variance associated with frontal deficits in AUD patients, observing that VEGFA has an essential role as a factor interacting with the pro-inflammatory immune response associated with alcohol consumption. Consistent with our results, using cellular and animal models, Muneer et al. (2012) found that chronic alcohol exposure disrupts the blood–brain barrier across degradation of endothelial VEGF receptor 2. This also increases circulating levels of VEGFA leading to neuronal death and inflammation in the brain [80]. Moreover, Louboutin et al. (2012) reported increases in VEGFA levels and blood vessel density in cerebral tissue within two weeks of the onset of ethanol consumption in rats [81]. Moreover, despite VEGFA not being an inflammatory cytokine, it can activate nuclear factor-enhancer of activated B-cell kappa light chains (NF-KB) and nuclear factor of activated T-cells (NFAT) signaling cascades that could promote a chemotaxis response involved in the angiogenic process (although this role remains unknown) [82]. Thus, our results in AUD patients with frontal deficits might suggest two things: (1) chronic alcohol abuse might lead to alterations in the concentrations of VEGFA that increase the permeability of the blood–brain barrier, leading to infiltration of immune cells and inflammation in the brain [80,81], and/or (2) under the presence of hypoperfusion and hypoxia as a result of alcohol-derived brain damage, concentrations of VEGFA might ultimately increase as a compensatory signal in order to form new blood vessels and recruit chemokines to the affected brain area. Lastly, it has been found that higher circulant levels of VEGFA in major depression and its alterations are related to impaired cognitive function in schizophrenia [83,84]. This might explain why psychiatric comorbidity affected plasma concentrations of VEGFA in our study. It is important to note that cognitive deficits found in patients with addictions can often be exacerbated by comorbid psychiatric disorders [85].

Lastly, previous studies have reported that fractalkine (CX3CL1) develops an essential role in the neuronal–microglial intercommunication [86]. This chemokine is expressed from brain neurons that control activation of microglia through its binding receptor CX3CR1. In harmful conditions, neurons release fractalkine in order to stimulate proliferation, activation and migration at the site of the brain injury [87,88]. CX3CL1-KO mice showed altered microglial function and neurotoxicity following LPS injection as well as more neuronal damage in Parkinson's disease and amyotrophic lateral sclerosis [88]. In accordance with this, Sokolowki et al. (2014) found that CX3CL1-KO mice revealed signals of neuronal apoptosis after ethanol treatment, suggesting a role in the clearance of those apoptotic cells [86]. Moreover, additional studies have reported that mild–moderate Alzheimer's disease patients had higher plasma levels of CX3CL1 than those with severe Alzheimer's disease [89,90]. These results suggest that fractalkine and CX3CR1 signaling might act as a neuroprotective mechanism through the microglial activity modulation in the early stage of brain injury while this signal seems to disappear when neuronal damage is established [91]. This may indicate that the AUD patients in this study are actively fighting against cognitive impairment.

5. Conclusions, Limitations, and Future Perspectives

In conclusion, a lifetime of chronic alcohol consumption leads to a proinflammatory systemic condition revealed by enhanced circulating chemokines, and to frontal cognitive impairment. The trophic factor VEGFA appears to be a relevant contributor to alcohol-associated neuroinflammation, probably through its role on controlling blood–brain barrier permeability, ultimately leading to impaired cognition. In addition, fractalkine could act as a signal of brain damage in early stages of cognitive impairment. Potential biomarkers could be useful and reliable tools in patients with AUD for confirming the diagnosis, defining the current stage of the AUD, and diagnosing these patients early.

Nevertheless, this study has several limitations that should be taken into account in future research. First, we do not know the time course of the effects of alcohol on these chemokines, either after acute or chronic alcohol consumption, nor its alterations in early or extended abstinence. We must also investigate whether sociodemographic variables, especially educational level, time of alcohol consumption, age of alcohol drinking initiation, etc., might contribute to the cognitive performance in both control and AUD patients. Finally, we lack significant representation of the female population, which precludes investigation of sex differences in chemokines and VEGFA. However, the data obtained clearly point to the need of considering these immunoinflammatory signals and trophic factors as relevant biomarkers of AUD-associated complications. Moreover, this concept should be extended to the analysis of immunomodulators capable of activating chronic inflammation. There are several biochemical pathways affected by alcohol consumption that need to be considered under the light of the present discoveries. For example, the tryptophan/kynurenine pathway is a potent immunomodulatory system that can modify inflammation, learning and memory [92,93]. The intestinal microbiota (which is determinant for AUD) [94] participates in these pathways, modifying the presence of pro- and anti-inflammatory mediators and eventually growth factors.

As a future perspective, we need to integrate all the information related to this multiplicity of inflammatory signals in a single model of alcohol addiction. Although certain factors such as VEGFA or fractalkine may contribute to important aspects of alcohol addiction, the complexity of the interactions of these inflammatory signaling proteins goes beyond our current technique and knowledge. Further clinical and technological research is necessary to elucidate the role of these factors in the etiology of AUD and associated comorbidities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines10050947/s1, Table S1: Plasma concentrations of chemokines and VEGFA in AUD group versus control subjects; Table S2: Correlation analysis between FAB score and chemokines in AUD patients; Table S3: Correlation analysis between plasma concentrations of chemokines and VEGFA in AUD patients with and without cognitive impairment; Table S4: Plasma concentrations of chemokines and VEGFA grouped according to comorbid psychiatric disorder; Table S5: Plasma concentrations of chemokines and VEGFA grouped according to comorbid substance use disorder; Table S6: Plasma concentrations of chemokines and VEGFA grouped according to comorbid medical problem; Table S7: Plasma concentrations of chemokines and VEGFA grouped according to the use of psychotropic medication last year.

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Conclusiones parciales del Estudio 3:

- Los pacientes TUA tienen mayores concentraciones plasmáticas SDF-1 y MCP-1 en comparación con los sujetos de control
- Los pacientes TUA con déficit en cognición frontal tuvieron mayores niveles de VEGFA que en aquellos sin deterioro cognitivo
- La administración aguda de una dosis alta de alcohol resultó en un aumento tardío (8 h después de la ingestión de alcohol) de concentración plasmática de VEGFA en sujetos sanos.
- Las funciones del lóbulo frontal se correlacionaron negativamente con VEGFA y fractalquina.
- Las concentraciones plasmáticas de VEGFA se correlacionaron fuerte y positivamente con todas las quimioquinas en pacientes TUA con déficit frontal pero no en aquellos sin deterioro frontal.
- Dos componentes juntos explicaron el 80,67% de la varianza asociada a los déficits frontales en pacientes TUA, actuando VEGFA como enlace entre todas las quimioquinas.
- Las concentraciones plasmáticas de algunas quimioquinas cambiaron en pacientes TUA con trastornos comórbidos psiquiátricos (MIP-1α, VEGFA) y por uso de sustancias (MIP-1 α).

4.4. Estudio 4:

Las concentraciones en plasma de la proteína de cadena ligera del neurofilamento y el factor neurotrófico derivado del cerebro son biomarcadores consistentes de deterioro cognitivo asociado con los trastornos por uso de alcohol

"Plasma concentrations of neurofilament light chain protein and brain-derived neurotrophic factor are consistent biomarkers of cognitive impairment associated to alcohol use disorders"

PLASMA CONCENTRATIONS OF NEUROFILAMENT LIGHT CHAIN PROTEIN AND BRAIN-DERIVED NEUROTROPHIC FACTOR ARE CONSISTENT BIOMARKERS OF COGNITIVE IMPAIRMENT ASSOCIATED TO ALCOHOL USE DISORDERS

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Running title: Biomarkers of alcohol-induced cognitive impairment

Keywords: Alcohol Use Disorder, Cognitive impairment, Neurofilaments, BDNF, Dementia.

ABSTRACT

Alcohol consumption has been proposed to be a relevant contributor to dementia. However, the possibility of tracking early neuronal toxicity associated to cognitive impairment in alcohol use disorder (AUD) patients is a major clinical challenge. Here we report that substance use disorder patients ((SUD, N=60) versus controls (N=40)), display an increase in plasma concentrations of neurofilament light chain (NFLs) and a decrease in plasma brain derived neurotrophic factor (BDNF). Both biomarkers were directly associated with AUD and to the onset and severity of cognitive impairment. Plasma levels of NfLs were also elevated in outpatients with dementia (N=27) and its concentration in AUD was associated with domains of executive function and memory cognition. Alterations in plasma concentrations of NfLs and BDNF were mediated by chronic alcohol abuse but not by lifetime of cocaine, cannabis consumption or affective disorders. Since NfL is a well-known biomarker for neurodegeneration, particularly in young individuals, and BDNF is a known marker of cognitive decline, we propose its combined use for monitorization of early cognitive impairment associated to AUD.

INTRODUCCION

Dementia is considered a public health priority because of his alarming increasing of prevalence [7.7 million per year], the huge economic impact and the overwhelming consequences for family and caregivers. It is estimated that dementia will affect 65.7 million of people in 2030 and 115.4 million in 2050. Thus, the WHO have endorsed a *global action plan on the public health response to dementia 2017-2025* for the development of a coordinated global response to cope with this disease effectively. One important approach is to control factors contributing to the development of dementia, including cardiovascular and dietary health, Interestingly, lifetime substance abuse has been associated with progressive cognitive decline and neuronal injury throughout life[1,2]). In special, alcohol addiction is being considering a factor that contributes to al known dementias[3]. However, except for the alcohol-abuse associated thiamine deficiency-induced dementia, alcohol consumption is not considered an essential component in the etiology of the disease.

Several population studies have proposed a U-shaped or J-shaped relationship between alcohol consumption and development of dementia[4,5,6]. Light-moderate alcohol consumption might decrease the risk of dementia, while heavy drinking could increase the risk[7,8] . Sabia et al[6] suggested that a consumption > 14 units / week increases the risk of dementia linearly with a consumption > 23 drinks / week (> 38 g / day) being the dose associated with the higher risk (W. Xu et al., 2017). In this line, an Australian study reported the most common clinical diagnosis for early-onset dementia were alcohol-related dementia (18.4%), followed by Alzheimer's disease (17.7%), vascular dementia (12.8%) and frontotemporal dementia (11.3%), being more frequent in ages between 45 to 64 years[9] . Similarly, a French study found that Alcohol Use Disorder (AUD) was the strongest risk factor for dementia onset in both men and women and the strongest association with alcohol brain damage was in the final stage of liver disease followed by liver cirrhosis[3]. However,

compared with research on cognitive impairment and dementia-related processes in alcohol, the background in cocaine and cannabis use disorder is still scarce and inconsistent[10,11] (Figueiredo et al., 2020; Frazer et al., 20. Even though there is no scientific literature linking cocaine dependence with the development of dementia, these patients manifest severe cocaine-induced cardiovascular consequences such as vasoconstriction, endothelial dysfunction, arteriosclerosis[12] and increases in oxidative stress[13] that could underlie cognitive dysfunction[12,14]. Moreover, acute cocaine use could also be a contributing factor to stroke risk in young people[15]. Similarly, a review of longitudinal studies suggests that although heavy cannabis use leads to neuropsychological impairment, these associations might be attenuated or not significant when confounding variables as other substance use, psychiatric disorders or psychosocial variables are controlled[16].

Chronic alcohol consumption can result in several neuropsychological alterations related to processing speed and sustained attention, learning and memory as well as global executive functions including disinhibition, cognitive flexibility, planification, resolution strategies and working memory, especially when processing visuospatial information[17-20]. These deficits are also related to changes in emotional processing and social cognition[21,22]. Additionally, comorbid mood and anxiety disorders may worsen certain cognitive domains and functional disability in these patients[23]. In this line, it is widely known that chronic alcohol consumption triggers an organic proinflammatory state that can affect the brain causing neurocognitive alterations[24-26]. Alcohol can stimulate Toll-like receptor 4 that activates several signaling pathways (NF-kB, MAPK, iNOS) resulting in the release of cytokines, chemokines and oxidative-nitrosative stress[16,27-28], which have been associated with neuroinflammation and structural brain damage[29,30]. Furthermore, the increase in inflammatory signals have been related to decreases in neuroprotective signals such as Brain Derived Neurotrophic Factor (BDNF) and the transcriptional Nrf-2 factor[16,31]. Recently, we have found that Neurotrophin-3 and BDNF, members of the neurotrophin

family of growth factors, could be implicated in compensatory mechanisms for cognitive impairment in the early stage of AUD[32]. These findings also suggested that plasma concentration of BDNF might be affected by the state of frontal lobe functions and monitoring BDNF signaling could help to identify AUD patients with and without cognitive impairment with high precision[32,33].

Therefore, detecting factors responsible for cognitive decline in patients at risk is of particular importance to initiate interventions at this stage that can delay or prevent the onset of dementia. In this way, Neurofilament Light chains (NfLs) are a structural component of the axonal cytoskeleton that is released into the blood and cerebral spinal fluid as a consequence of neuronal cell injury[34]. Thus, it has been proposed as a biomarker for neurodegeneration in several neurodegenerative diseases such as mild cognitive impairment, Alzheimer's disease, frontotemporal dementia, Parkinson's disease, Down syndrome and amyotrophic lateral sclerosis[35]. It also has provided superior capabilities to discriminate neurodegenerative disease in younger (<65 years) tan in older (>65 years) individuals[35]. Hence, NfLs have promise because they revealed structural brain damage in peripheral blood plasma by ultrasensitive analytic platforms[36] being a less invasive technique than cerebral spinal fluid assessments and less expensive than neuroimage diagnostics.

Since clinical studies have recognized NfLs as a promising biomarker for early-onset of alcohol-related dementia, we further explore whether NfLs are affected in patients with substance use disorders (SUD) [mainly with AUD]. Thus, the main aim of this descriptive clinical study was to characterize plasma concentrations of NfLs in substance dependent patients exploring its association with cognitive deficits and addiction-related variables. Furthermore, we investigated the interaction of NfL and BDNF, a well described biomarker for cognitive impairment in this population. The ultimate goal is to establish

whether alcohol-associated early cognitive deficits are associated with not only trophic but active neurodegenerative process measurable by objective peripheral biomarkers.

RESULTS

Sociodemographic characteristics

Table 1 shows a socio-demographic description of the total sample. We recruited 60 patients in abstinence from substance use outpatient programs, 27 patients from neurology outpatient settings and 67 healthy control subjects. The mean age of the SUD group was 41 years and the 83% of the participants were men with a BMI index of 26.47 kg/m^2, while the mean age of the dementia group was 75 years and the 55% were men with a BMI of 25.79 kg/m^2. Because of the huge differences between SUD group and dementia group, we could not match the three groups in age (p<0.001) and sex (p=0.001), unless by BMI. However, control group was selected as being as similar as possible between the SUD group and the dementia group. Significant differences were observed between the three sample groups with respect to educational level (p=0.002) and occupation (p<0.001).

Table 1. Socio-demographic characteristics of the sample.

TOTAL SAMPLE N=127					
VARIABLES		**Control group N=40**	**SUD group N=60**	**Dementia group N=27**	***p* value**
Age *(Mean±SD)*	*Years*	52.25 ± 2.28	41.37 ± 12.40	75.33 ± 5.41	**<0.001**[1]
Body Mass Index *(Mean±SD)*	*Kg/m^2*	27.71 ± 4.05	26.47 ± 5.74	25.79 ± 3.15	0.320[2]
Sex *[N (%)]*	*Women* *Men*	20 (50) 20 (50)	10 (16.70) 50 (83.30)	12 (44.40) 15 (55.60)	**0.001**[3]
Education Level* *[N (%)]*	*Low* *Medium* *High*	14 (35) 12 (30) 13 (32.50)	20 (35) 28 (46.70) 11 (18.30)	19 (70.40) 6 (22.20) 1 (3.70)	**0.002**[3]
Occupation* *[N (%)]*	*Employed* *Unemployed* *Retired* *Sick leave*	33 (82.50) 3 (7.50) - -	17 (28.30) 27 (45) 11 (18.30) 5 (8.30)	1 (3.70) - 24 (88.9) -	**<0.001**[3]

	Housework	4 (10)	-	1 (3.70)	

[1] value was calculated with Kruskal-Wallis's test. [2] value was calculated with ANOVA's t test. [3] value was calculated with Fischer's exact test or chi-squared test. Bold values are statistically significant for $p<0.05$. (*) There were lost data in the educational level variable (one control participant and one dementia patient) and in the occupation variable (one dementia patient).

Addiction-related variables in SUD group

The variables related to the SUD group were evaluated and described in Table 2. Among patients who attended outpatient treatment for substance abuse; alcohol, cocaine and cannabis use disorders were the most prevalent with a 78%, 60% and 41% of the total SUD group, respectively. The average age at the time of the first beverage consumption was 14 years, while the average age of the AUD onset was 18 years with 17 years of problematic alcohol use. Similarly, the mean age at first consumption of cannabis was 15 years and the age of cannabis use disorder onset was 18 years with 13 years of problematic cannabis use. However, the mean age at first use of cocaine was 19 years and the average age of the cocaine use disorder onset was 25 years with 11 years of cocaine misuse. The mean of severity criteria of addiction of these three substances was 6 approximately based on DSM-IV criteria [1-11] and the mean duration of abstinence was more than one year. Finally, we observed a high prevalence of other comorbid SUD (60%) in AUD patients, especially with cocaine (48%) and cannabis (27%) use disorders.

In addition, there was an elevated prevalence of other comorbid psychiatric disorders (76.7%), showing lifetime mood and anxiety disorders the most frequent, diagnosed in a 50% and 35%, respectively. Furthermore, the 85% of the abstinent SUD patients received psychiatric medication during the last year being more common the prescription of antidepressants (43%), anxiolytics (57%) and disulfiram (18%). The neuropsychological evaluation revealed that a 57% of the SUD group showed some deficits related to global cognition (assessed by MoCA).

Table 2. Clinical characteristics of the SUD group.

VARIABLES			SUD group N=60
Substance use disorders *[N (%)]*		*Alcohol*	47 (78.30)
		Cocaine	36 (60)
		Cannabis	25 (41.70)
		Sedatives	8 (13.30)
		Opioids	7 (11.70)
		Other comorbid SUD	36 (60)
Age at first drug use *[Mean (SD)]*	*Years*	*Alcohol*	14.04 (4.31)
		Cocaine	19.22 (5.05)
		Cannabis	14.60 (2.48)
		Sedatives	18.50 (1.77)
		Opioids	20.86 (8.44)
Age at onset of SUD *[Mean (SD)]*	*Years*	*Alcohol*	17.85 (9.31)
		Cocaine	24.75 (8.38)
		Cannabis	16.92 (4.04)
		Sedatives	24.25 (13.46)
		Opioids	22.14 (7.99)
Length of SUD diagnosis *[Mean (SD)]*	*Years*	*Alcohol*	16.70 (12.06)
		Cocaine	10.93 (8.65)
		Cannabis	13 (10.01)
		Sedatives	7.88 (5.94)
		Opioids	14.57 (13.54)
Severity criteria *[Mean (SD)]*	*Criteria [1-11]*	*Alcohol*	6.35 (2.91)
		Cocaine	6.11 (3.66)
		Cannabis	5.73 (3)
		Sedatives	7 (2.98)
		Opioids	4 (4.36)
Length of abstinence *[Mean (SD)]*	*Days*	*Alcohol*	400.24 (995.31)
		Cocaine	216.67 (672.13)
		Cannabis	526.68 (1488.22)
		Sedatives	72.43 (167.31)
		Opioids	1103.43 (1290.84)
Comorbid psychiatric disorders *N (%)]*		*Mood*	31 (51.70)
		Anxiety	21 (35)
		Personality	12 (20)
		Psychotic	5 (8.30)
Psychiatric medication *[N (%)]*		*Antidepressants*	26 (43.30)
		Anxiolytics	34 (56.70)
		Antipsychotics	9 (15)
		Anticraving	5 (8.3)
		Disulfiram	11 (18.30)
Cognitive impairment (MoCA) *[N (%)]*		*No*	26 (43.3)
		Yes	34 (56.7)

Plasma concentrations of NfL and BDNF in the total sample.

The impact of substance abuse on plasma concentrations of NfL and BDNF was studied in the total sample using a one-way ANCOVA with "group" (SUD group, dementia group and control group) as factor and age as covariate. Plasma concentrations of NfLs were significantly affected by "group" factor ($F_{2,123}$=16.409, p<0.001, η_p^2=0.211). NfLs plasma concentrations were significantly increased in the SUD group (p=0.001) and the dementia group (p<0.001) when they were compared with the control group (Fig 1A). Moreover, plasma concentrations of BDNF were significantly affected by "group" factor ($F_{2,97}$=16.264, p<0.001, η_p^2=0.251). Thus, plasma concentrations of BDNF were decreased in the SUD group (p<0.001) than the control group. However, there were no differences in the dementia group (Fig 1B).

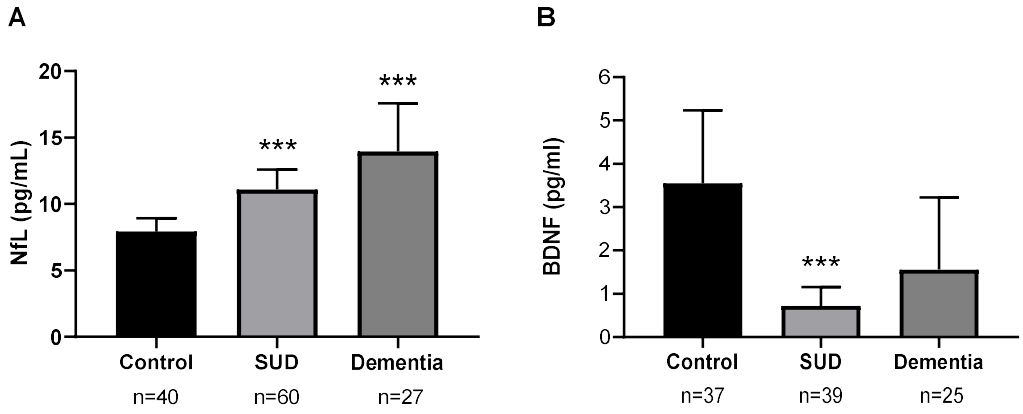

Figure 1. Plasma concentrations of NfL and BDNF according to the group. A) Bars are estimated marginal means and 95% confidence intervals representing NfL concentrations (pg/mL) according to the group. B) Bars are estimated marginal means and 95% confidence intervals representing BDNF concentrations (pg/mL) according to the group. Data were analyzed by one-way analysis of covariance (ANCOVA) and ***p < 0.001 denote a significant main effect of group factor.

Correlation of plasma concentrations of NfLs with BDNF according to the group in the whole sample of subjects

Correlation analyses using Spearman correlation (rho) were performed with the plasma concentrations of NfLs and BDNF in the SUD group, the dementia group and control group. Thus, the statistical analyses found a negative correlation between plasma concentrations of NfLs and BDNF in the SUD group (rho=-0.485, p=0.002) and the control group (rho=-0.344, p=0.037). Interestingly, we did not find significant correlations between NfL and BDNF in the dementia group (rho=-0.159, p=0.448) (Supplementary Fig 1).

Plasma concentrations of NfL/BDNF ratio in the whole sample of subjects.

We examined the ratio of NfL/BDNF in the total sample to clarify the relationship between NfL and BDNF signaling using a one-way ANCOVA with "group" (SUD group, dementia group and control group) as factor and age as covariate**.** The NfL/BDNF ratio was significantly affected by "group" factor ($F_{2,97}$=22.326, p<0.001, η_p^2=0.315). The NfL/BDNF ratio was significantly increased in the SUD group (p<0.001) and the dementia group (p=0.010) when they were compared with the control group (Fig 2).

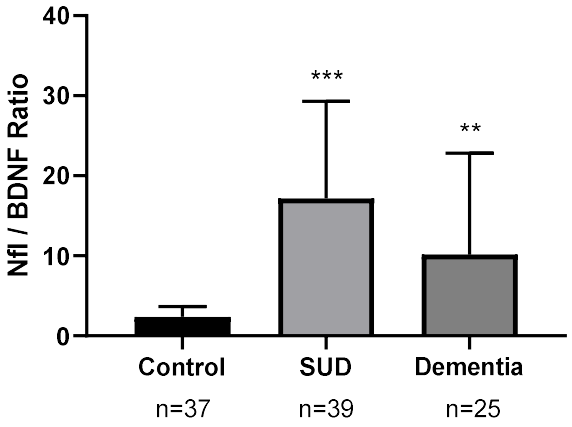

Figure 2. BDNF/NfL ratio according to the group. A) Bars are estimated marginal means and 95% confidence intervals representing the BDNF/NfL ratio (pg/mL) according to group. Data were analyzed by one-way analysis of covariance (ANCOVA). ***p < 0.001 and **p<0.010 denote a significant main effect of the group factor.

Plasma concentration of NfLs and BDNF in cognitive impairment in abstinent SUD patients

The neuropsychological evaluation in the SUD group revealed that the 28% of the showed mild cognitive deficits, the 22% revealed moderate cognitive deficits and the 7% demonstrate signs of severe cognitive impairment related to global cognitive functions assessed by MoCA's test.

We measured cognitive functioning following chronic substance consumption in plasma concentrations of NfLs and BDNF in SUD group (assessed by MoCA's) using a one-way ANCOVA with "cognitive impairment" (non-cognitive impairment, mild cognitive impairment and moderate/severe cognitive impairment) as a factor and age as covariate. Interestingly, plasma concentrations of NfLs were significantly affected by "cognitive impairment" factor ($F_{2,56}$=3.545, p=0.036, η_p^2=0.112). There were significantly more plasma concentrations of NfLs in SUD patients with moderate-severe cognitive impairment (p=0.032) than in those without cognitive impairment. However, we did not observe main differences in mild cognitive impairment (Fig 3A). Furthermore, plasma concentrations of BDNF were significantly affected by "cognitive impairment" factor ($F_{2,34}$=7.284, p=0.002, η_p^2=0.294). Thus, SUD patients with moderate-severe impairment had augmented plasma concentrations of BDNF (p=0.002) than those with non-cognitive impairment. However, we did not observe main differences in mild cognitive impairment (Fig 3B).

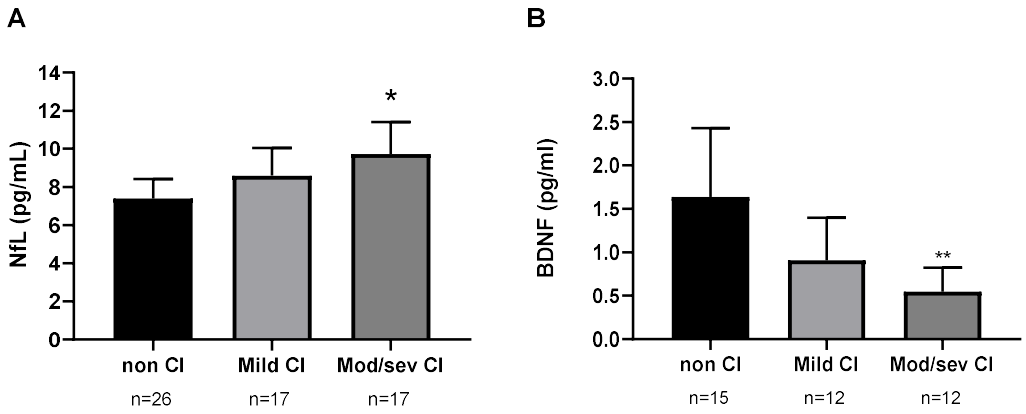

Figure 3. Plasma concentrations of NfL and BDNF according to cognitive impairment in SUD patients. A) Bars are estimated marginal means and 95% confidence intervals representing NfL concentrations (pg/mL) according to the cognitive impairment. B) Bars are estimated marginal means and 95% confidence intervals representing BDNF concentrations (pg/mL) according to the cognitive impairment. Data were analyzed by one-way analysis of covariance (ANCOVA). *$p < 0.05$ and **$p < 0.01$ denotes a significant main effect of cognitive impairment factor. CI= cognitive impairment.

Correlation analyses of NfLs and BDNF with tests of memory and executive functions in SUD patients.

We investigated cognitive functioning following chronic substance consumption, in memory and executive function (measured by TAVEC, ROCF, TMT B and Digit Span Subtest) in plasma concentrations of NfLs and BDNF in the SUD group with cognitive impairment (assessed by MoCA).

Table 4 shows the psychometric data obtained by the SUD group in each neuropsychological test. The Z scores of the *immediate free recall in the list A* was -1.30 ± 1.0 and in the *immediate free recall in the list B* was -1.09 ± 0.631, showing mild deficits in verbal learning. Furthermore, Z scores of s*hort delay free recall, short delay cued recall, long delay free recall and long delay cued recall* were -1.39 ± 1.200, -1.30 ± 0.984, -1.03 ± 1.045, -1.18 ± 1.310, which demonstrated mild alterations in short and long-term verbal memory that could not be prevented through semantic cues. The Z score of *Digit span subtest* was -1.18 ± 1.468, suggesting mild impairment in the phonological loop. The Z

score of the *TMT B* was 3.14 ± 2.74, indicating severe deficits related to impairment in executive attention and mental flexibility.

Moreover, as shown in Table 4, there were significant and negative correlations between NfLs and variables associated with interference [*immediate free recall list B* (TAVEC)], planification [*serial strategies in immediate recall* (TAVEC) and *serial strategies in short delay free recall* (TAVEC)] and slightly with verbal short-term memory [*short delay free recall* (TAVEC)*].* We observed significant and positive correlation between NfLs and memory intrusions [*intrusions in free recall, intrusions in cued recall* (TAVEC)]. Finally, we found a slight positive correlation between NfLs and a variable associated with attentional executive function [*Time* (TMT B)]. However, we did not find correlations between BDNF and scores of cognitive assessments. We only found a slight positive association between BDNF and visual short-term memory [*memory* (ROCF)].

Table 4. Neuropsychological psychometric values and its correlations with plasma concentrations of NfL and BDNF in SUD patients.

VARIABLES		**SUD group with cognitive impairment N=34**			
		Psychometric data		**Correlation Analyses**	
		Direct score [Mean (SD)]	**Z score [Mean (SD)]**	**NfL [Rho (*p* value)]**	**BDNF [Rho (*p* value)]**
	Immediate free recall (list A)	37.09	-1.30*	-279 (0.166)	0.058 (0.792)
	Immediate free recall (list B)	4.42	-1.09*	**-0.363 (0.038)**	0.271 (0.211)
	Short delay free recall	8.42	-1.39*	-0.316 (0.073)	0.347 (0.104)
	Short delay cued recall	9.88	-1.30*	0.225 (0.209)	0.312 (0.147)
	Long delay free recall	10.06	-1.03 *	0.000 (0.999)	0.197 (0.367)
	Long delay cued recall	10.36	-1.18*	0.095 (0.598)	0.049 (0.825)
	Semantic strategies in immediate recall	8.91	-0.76	-0.251 (0.159)	0.208 (0.342)
	Semantic strategies in short delay free recall	2.36	-0.76	-0.135 (0.455)	0.010 (0.965)

	Semantic strategies in long delay free recall	3.27	-0.70	-0.88 (0.626)	-0.038 (0.863)
	Serial strategies in immediate recall	2.94	-0.45	**-0.374 (0.032)**	0.215 (0.325)
	Serial strategies in short delay free recall	0.30	-0.42	**-0.464 (0.007)**	0.143 (0.516)
	Serial strategies in long delay free recall	0.15	-0.55	-0.190 (0.290)	0.000 (1)
	Recognition	14.30	-0.33	-0.158 (0.380)	0.293 (0.175)
	Intrusions in free recall	5.48	0.61	**0.408 (0.018)**	-0.083 (0.707)
	Intrusions in cued recall	3.12	0.73	**0.362 (0.038)**	-0.214 (0.327)
	Perseverations	3.58	-0.42	-0.266 (0.135)	0.126 (0.565)
ROCF	*Time (minutes)*	2.97	-0.154	0.209 (0.297)	-0.206 (0.383)
	Figure	33.80	0.96	-0.297 (0.124)	-0.058 (0.807)
	Memory	19.35	-0.10	-0.306 (0.121)	0.402 (0.088)
TMT B	*Time (seconds)*	106.26	3.37**	0.349 (0.074)	-0.315 (0.190)
DIGITS SPAN SUBSTEST	*Direct digits span*	4.89	-1.18*	-0.131 (0.516)	-0.218 (0.371)
	Backward digits span	4.15	-0.49	-0.202 (0.311)	-0.309 (0.198)

(*) Values show Z scores below -1 as the cutoff for mild cognitive impairment. (**) Value shows Z score below -2 as the cutoff for severe cognitive impairment. Bold values are statistically significant for $p<0.05$.

Plasma concentrations of NfLs and BDNF according to the substance of abuse in SUD patient

As shown in the clinical description of the sample (Table 2), alcohol, cocaine, and cannabis use disorders had an elevated prevalence among SUD patients. Moreover, we observed high rates of SUD comorbidity, especially between alcohol and cocaine/cannabis use disorders. Thus, we examined the effect of each condition in circulants levels of NfL comparing with the control group using a one-way ANCOVA with “group” [alcohol group, cocaine/cannabis group, comorbid groups (alcohol plus cocaine/cannabis) and control group] as factor and age as covariate.

Regarding alcohol and cocaine substances, plasma concentrations of NfLs were significantly affected by "group" factor ($F_{3,93}$=6.335, p=0.001, η_p^2=0.170). There were significantly higher plasma concentrations of NfLs in the alcohol group (p=0.003) and comorbid group (alcohol plus cocaine, p=0.005) compared with the control group. However, we did not find main differences on NfLs concentrations in the cocaine group. Estimated means an 95% CI for "group" factor are represented in Fig 4A. Similarly, plasma concentrations of BDNF were significantly affected by "group" factor ($F_{3,71}$=15.869, p<0.001, η_p^2=0.401). Plasma concentrations of BDNF were decreased in the alcohol (p<0.001) and comorbid group (alcohol plus cocaine, p<0.001) than in the control group. However, we did not find main differences on BDNF concentrations in the cocaine group. Estimated means an 95% CI for "group" factor are represented in Fig 4B.

Concerning alcohol and cannabis, plasma concentrations of neurofilaments were significantly affected by "group" factor ($F_{3,91}$=5.914, p=0.001, η_p^2=0.163). NfLs plasma concentrations were significantly increased in the alcohol group (p=0.004) and comorbid group (alcohol plus cannabis, p=0.004) compared with the control group. However, we did not observe main differences on NfLs concentrations in the cannabis group. Estimated means an 95% CI for "group" factor are represented in Fig 4C. Accordingly, plasma concentrations of BDNF were significantly affected by "group" factor ($F_{3,67}$=14.743, p<0.001, η_p^2=0.397). Plasma concentrations of BDNF were decreased in the alcohol (p<0.001) and comorbid group (alcohol plus cannabis, p=0.003) than in the control group. However, we did not find main differences on BDNF concentrations in the cannabis group. Estimated means an 95% CI for "group" factor are represented in Fig 4D.

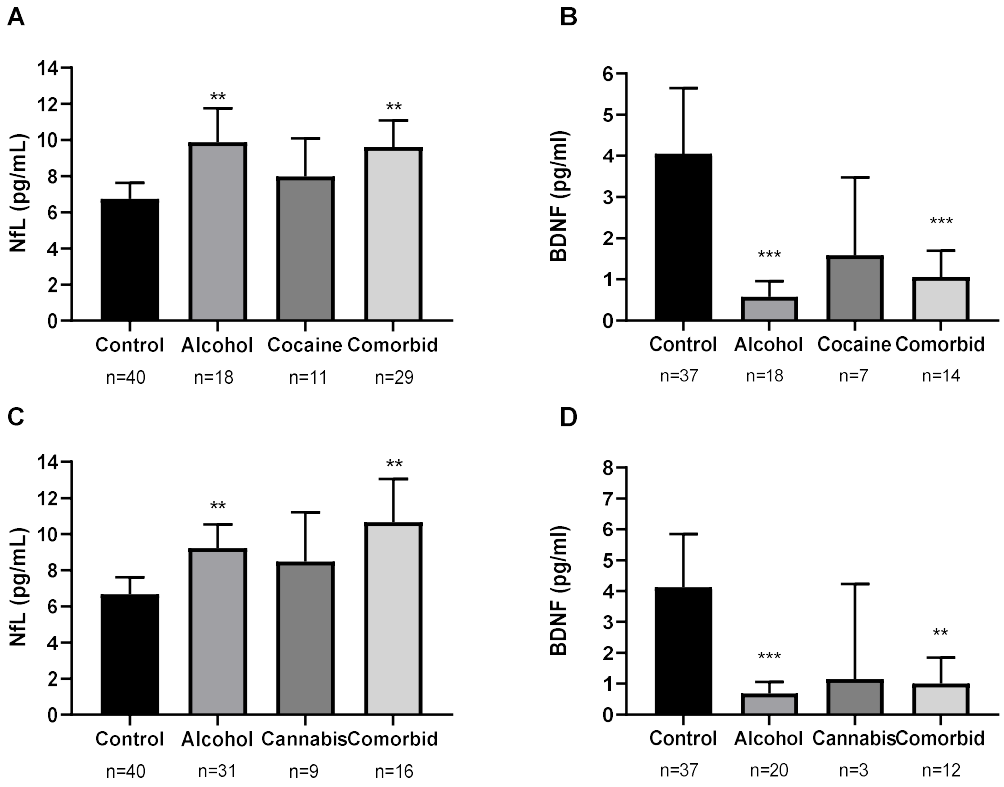

Figure 4. Plasma concentrations of NfLs and BDNF according to type of drug use. A) Bars are estimated marginal means and 95% confidence intervals representing NfLs concentrations according to alcohol and cocaine drug use. B) Bars are estimated marginal means and 95% confidence intervals representing BDNF concentrations (pg/mL) according to alcohol and cocaine drug use. C) Bars are estimated marginal means and 95% confidence intervals representing NfLs concentrations (pg/mL) according to alcohol and cannabis drug use. D) Bars are estimated marginal means and 95% confidence intervals representing BDNF concentrations (pg/mL) according to alcohol and cannabis drug use. Data were analyzed by one-way analysis of covariance (ANCOVA) and $^{**}p < 0.01$ and $^{***}p < 0.001$ denotes a significant main effect of type of drug use.

Correlation analyses of NfLs and BDNF with addiction-related variables according to the type of drug use in SUD patients

Moreover, correlation analyses using Spearman correlation (rho) were performed with the plasma concentrations of NfLs and BDNF with addiction-related variables according to the type of drug use. The statistical analyses found a positive correlation between plasma concentrations of NfLs and the age at onset of AUD (age, rho=0.451,

p=0.001), and a positive correlation between plasma concentrations of NfLs and the length of AUD diagnosis (years, rho=0.375, *p*=0.010). Additionally, we found a negative correlation between plasma concentrations of BDNF and age at onset of AUD (age; rho=-0.419, *p*=0.017), and a negative correlation between plasma concentrations of BDNF and alcohol severity (criteria 1-11, rho=-0.351, *p*=0.049) (Supplementary Fig 2). However, we did not observe significant correlations between plasma concentrations of NfLs and BDNF with the remaining addiction-related variables according to the type of drug.

Plasma concentrations of NfLs and BDNF in comorbid psychiatric disorders in abstinent SUD patients.

As shown in the clinical description of the sample (Table 2), mood and anxiety disorders were the most prevalent comorbid psychiatric disorders in SUD patients. Thus, we examined the effect of mood and anxiety comorbid disorders on plasma NfLs and BDNF in the SUD group using a one-way ANCOVA with "anxiety/mood disorder" (comorbid group and non-comorbid group) as a factor and age as covariate. However, we did not observe main differences on NfLs and BDNF concentrations in comorbid anxiety ($F_{1,57}$=2.903, *p*=0.094; $F_{1,36}$=0.011, *p*=0.918, respectively) and mood disorders ($F_{1,57}$=0.047, *p*=0. 830; $F_{1,36}$=0.044, *p*=0.834, respectively) in SUD patients.

Cognitive impairment-based prediction variables in SUD patients

Finally, we generated a binary logistic regression model to evaluate the potential of BDNF, NfL and alcohol-related variables as exploratory variables to discriminate between SUD patients with and without cognitive impairment. The variables included in the first step were "age", "NfL", "BDNF", "age at onset of AUD", "length of AUD diagnosis" and "alcohol severity". As a result, Hosmer-Lemeshow test indicated good calibration (Chi-squared test=14.197; *p*=0.077) and was able to explain the variation of the dependent variable in 50.4% of the cases according to the Nagelkerke R2 method. It had a classification percentage of 90.6%, showing a high sensitivity for classifying SUD patients

with cognitive impairment (95%) and without cognitive impairment (83.3%). As seen in Fig 4, the ROC curve analysis indicated an AUC=0.946, which represented high discrimination power (Fig 4A). The scatter plot of the predictive probabilities for the SUD patients indicated that the means were significantly different between both groups (U=13, $p<0.001$) (Fig 4B).

Then, we performed a binary regression analysis to evaluate the potential of plasma NfL and BDNF alone to be good predictors for discriminating between SUD patients with and without cognitive impairment. The variables included in the first step were “age”, “NfL” and “BDNF”. As a result, Hosmer-Lemeshow test indicated good calibration (Chi-squared test=5.020; $p=0.755$) and was able to explain the variation of the dependent variable in 44.8 % of the cases according to the Nagelkerke R2 method. It had a classification percentage of 79.5%, showing a high sensitivity for classifying SUD patients with cognitive impairment (87.5%) and a low sensitivity for those without cognitive impairment (66.7%). As seen in Fig 4, the ROC curve analysis indicated an AUC=0.844, which represented high discrimination power (Fig 4C). The scatter plot of the predictive probabilities for the SUD patients indicated that the means were significantly different between both groups (U=56, $p<0.001$) (Fig 4D).

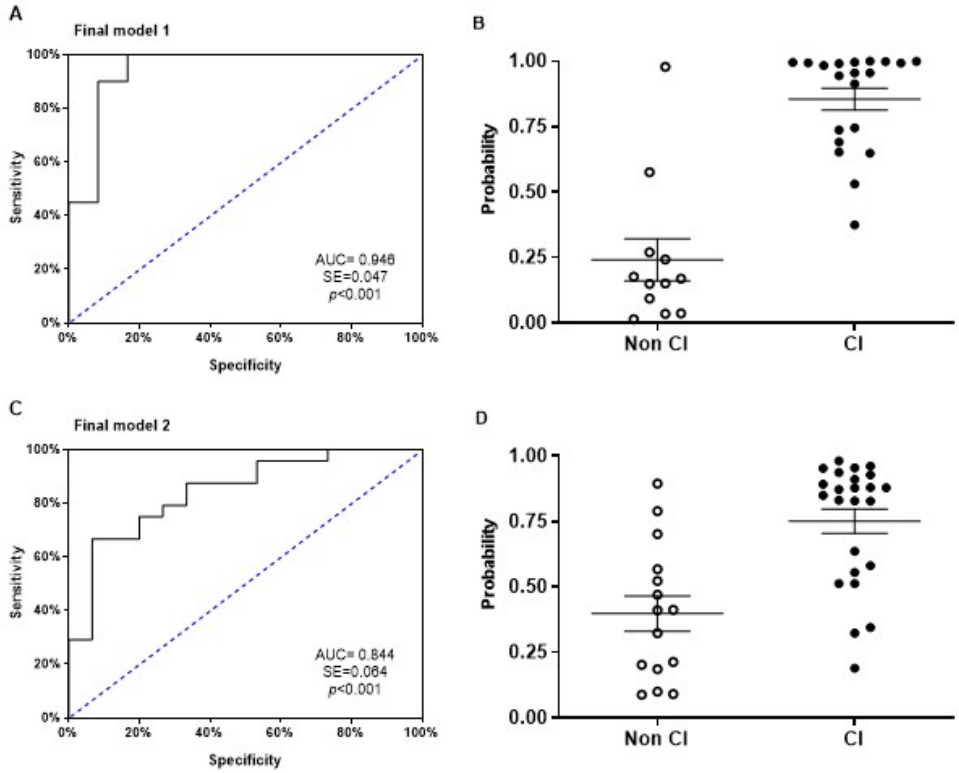

Figure 5. ROC analyses and scatter dots for multivariate predictive of final models of cognitive impairment. A) ROC curve for the final model 1 whose introduced variables introduced were: "age", "NfL", "BDNF", "age at onset of AUD", "length of AUD diagnosis" and "alcohol severity". B) Scatter plot of the predictive probabilities for the final model 1. (C) ROC curve for the final model 2 whose introduced variables introduced were: "age", "NfL" and "BDNF". D) Scatter plot of the predictive probabilities for the final model 2. The lines of the scatterplots are means and standard deviations. CI= cognitive impairment.

DISCUSION

The present study describes how plasma concentrations of NfLs is a reliable predictive biomarker of cognitive impairment in a population of SUD patients. It supports empirical evidence that alcohol abuse throughout life can be compared as a progressive neurodegenerative disease that triggers an early onset of cognitive impairment, the firt step towards dementia. Moreover, the analysis reveals that this cognitive impairment is no only associated with plasticicty/trophic factors deficits, but to a real structural brain damage induced by alcohol. In addition, we highlight the role of BDNF as a compensatory

mechanism against neurodegeneration in early but not in advanced stages, revealing the possibility of identifying different stages of the progression of the disease that might helpt to stratify diagnostics and to select interventions.

The main results of this study indicated that: 1) plasma concentration of NfLs were higher in the SUD group and the dementia group than in the control group, while plasma concentrations of BDNF were lower in the SUD group compared with the control group, 2) there was a negative correlation between NfLs and BDNF in the SUD group and the control group but not in the dementia group, 3) the BDNF/NfL ratio was higher in the SUD group and the dementia group than in the control group, 4) augmented plasma levels of NfLs and decreased plasma levels of BDNF were observed in SUD patients with moderate-severe cognitive impairment compared with those without cognitive impairment, 5) plasma levels of NfLs were associated with domains of executive function and memory cognition, 5) changes in plasma concentrations of NfLs and BDNF were mediated by chronic alcohol abuse but not by lifetime of cocaine and cannabis consumption, 6) there were positive correlations between NfLs and age at onset of AUD and years of duration of AUD, whereas there were negative correlations between BDNF and age at onset of AUD and the severity of alcohol consumption, 7) plasma levels of NfLs and BDNF were not affected by comorbid mood and anxiety disorders, 8) plasma concentrations of NfL and BDNF and alcohol-related variables are good predictors for discriminating between SUD patients with and without cognitive impairment with high accuracy (94%).

Elevated concentration of NfL is a strong biomarker for neurodegeneration and is closely related with senescence[34]. Although NfL might be unspecific for distinguishing cognitive disorders like β-amyloid and tau in Alzheimer's disease[37], it has proven useful in differentiating certain neurological disorders that could be misdiagnosed. Thus, plasma NfL has demonstrated high accuracy in dissociating atypical parkinsonian disorder from Parkinson disease (86%-95%), Down Syndrome from Down Syndrome with Alzheimer's

disease (91%), and Amyotrophic lateral sclerosis (>90%) and frontotemporal dementia (>80%) from controls[35]. NfL concentration also has been proposed to be a potential measure to assess whether a neurodegenerative process is ongoing or is a consequence of psychiatric disorders such as depression or bipolar disorder. In this sense, the early diagnosis of frontotemporal dementia might be a challenge because its symptoms are overlapping with several symptoms of primary psychiatric disorders[38]. However, plasma NfLs are able to detect frontotemporal dementia from depression with high accuracy (98%)[35,38]. In addition, the elevation of NfLs in amyotrophic lateral sclerosis and frontotemporal dementia are the highest in comparison to other neurological diseases and its levels in cerebrospinal fluid are associated with progression, survival and disease severity[39]. In our study, not only there were augmented plasma concentrations of NfLs in the SUD group when was compared with the control group, but also the SUD group did not differ statistically with the dementia group. This implies direct evidence that SUD patients (particularly those with alcohol abuse) are under an early neurodegenerative process, as many epidemic studies have pointed out[4-6], that have similar evolution in other kinds of dementia. Thus, we only observe this effect on NfL levels when SUD patients were under moderate-severe cognitive impairment but not in mild stages. In this line, a longitudinal twin study found that moderate-elevated consumption of alcohol increased 57% the risk of dementia compared with the twin with light consumption and heavy consumption was associated with the development of dementia 5 years earlier[7]. However, a systematic review informed that the prevalence of alcohol-related dementia is not homogeneous, ranged from 8.70 per 100,000 to 25.6% in several studies[40], with men being the most predominant gender and the proportion of early-onset alcoholic dementia more consistent. Thus, a possible explanation of this heterogeneity in the population data is that the incidence of alcohol-related dementia and the risk derived to alcohol consumption might be underestimated and biased due to the high death cases in this kind of patients that were not

taking into account in these studies[41]. Therefore, despite being several pieces of evidence supporting the independent association between alcohol consumption and the etiology of dementia processes, its impact on the human brain is still underestimated. On the other hand, our results are in contrast with the findings of Karoly et al (2021)[42] in which they did not observe a direct association between plasma NfL and heavy alcohol use, but they found a significant negative relationship between grey matter thickness and circulating NfLs. Nevertheless, this study was performed in healthy individuals rather than in patients from addiction outpatient settings as in our study. Lastly, our results reveal that neuronal injury and cognitive impairment are mediated by direct effects of alcohol in the system and not by mood or anxiety disorders as previous studies reported.

On the other hand, growth factors are a set of proteins that play a crucial role in cell growth, proliferation and differentiation in the CNS and are important to cognitive processes[43]. Among them, the functions attributed BDNF include the regulation of neurogenesis, synaptogenesis and gliogenesis and the control of long-term potentiation (LTP) mechanisms in the hippocampus that result in improved memory and cognition[44]. In the present study, we found decreased plasma levels of BDNF in SUD patients compared to healthy controls, especially in those with mild-severe cognitive impairment which is in line with our previous findings in AUD patients demanding treatment[33,45]. Moreover, in another study we showed that circulating levels of BDNF along with specific alcohol-related variables are capable of discriminating between alcohol dependent patients with cognitive impairment and without cognitive impairment with high accuracy (88.2%)[32]. However, although reductions of peripheral BDNF have been widely reported in Alzheimer's disease and mild cognitive impairment[46,47], we did not find significant differences in the plasma level of BDNF in the dementia group. This can be explained by the fact that our study had a low sample size and a high heterogeneity (there were not only Alzheimer's disease patients)

in the dementia group. Nevertheless, it is important to note that BDNF also changes during age[48] and these studies did not adjust this variable in their analysis.

Furthermore, in the present study we wanted to evaluate the levels of NfLs and BDNF in parallel with the aim of investigating its interactive effects across different stages of cognitive impairment. Thus, we also found a negative correlation between plasma concentrations of NfLs and circulating levels of BDNF in the control group and SUD group but not in the dementia group. These results indicate that BDNF might be involved in a compensatory mechanism against neuronal damage in early but not in advanced stages of cognitive impairment when the disability is established. In addition, we observed that the NfL/BDNF ratio was higher in both, the SUD group and the dementia group than in the control group. This index might be of clinical significance since it combines the measure of the combined effect of a progressive loss of the trophic action of BDNF, with the progressive neuronal loss associated with release of Nfl, helping to establish an objective biological marker for cognitive decline. In other words, lifetime of alcohol consumption is not only toxic, but also anti-regenerative. According to our results, interventions in early stages of alcohol-use disorder that can enhance the BDNF/TrKB signaling pathway, might help to counteract the neurotoxic effects, but this effect is lost at later stages when neuronal damage/death reaches an irreversible stage. A similar idea has been proposed for compensatory responses that postpone the onset of symptomatology in early stages of Alzheimer's disease but cannot counteract neuropathology in more advanced stages[49-51]. In accordance, we have observed that cognitive reserve, neurotrophin-3 and BDNF might be compensatory mechanisms for brain damage in the early stage of AUD, but not in later phases[32].

Because our SUD sample displayed a high prevalence of alcohol, cocaine and cannabis use disorders, we also evaluated the existence of a possible association between circulating NfL levels and the type of drug dependence. Thus, we found that only alcohol

abuse modulated plasma concentrations of NfL (and BDNF) in the SUD group. Concordantly, a meta-analytic study suggest that actual evidence does not support the view that chronic cocaine use is associated with wide cognitive deficits, despite the fact that brain and metabolic differences have been found in these patients[11]. Although some studies suggest that prolonged cocaine use causes deterioration in some cognitive domains, these could be due to the concomitant use of alcohol as our study supports. Similarly, more research is needed to determine if there are cognitive impairments associated with chronic cannabis use[10]. Whereas some studies report a long-term neuropsychological decline derived from neurotoxic effects of cannabis[52], other investigations suggest that cannabis use in the adolescence not seem to decrease intelligence quotient or executive function except for spatial working memory[53]. However, several studies exhibit a wide variety of degrees in cognitive dysfunction in alcohol dependent patients, ranging from mild cognitive impairment to dementia, especially early onset[3,20]. The 56% of our patients were diagnosed with cognitive impairment to some degree. They presented mild alterations in verbal learning, verbal short and long-term memory, phonological loop and severe deficits in executive attention and mental flexibility as reported in other investigations[18,19] .That neuropsychological alterations have also been related with greater difficulties in maintaining abstinence and moderate alcohol consumption[54,55]). Furthermore, we found negative correlations between circulant levels of NfL and interference, planification and verbal short-term memory, and a positive relationship with memory intrusions. Thus, we have demonstrated that neuronal structural damage under cognitive deficits is related to AUD. This is in line with a recent meta-analysis that has revealed grey matter degeneration in the right cingulate gyrus, left middle frontal gyrus and right insula in alcohol dependent patients, and this last was associated with the duration of abstinence[56]. Lifetime alcohol abuse has also been consistently associated with white matter reduction in several regions that implies frontal connections[57,58]. Moreover, this study also highlights the permanent and

ascendent nature of brain damage derived from AUD throughout life. Thus, we show that plasma concentrations of NfL increase according to the duration of AUD through life and the BDNF compensatory mechanism decay with the progressive severity increase of alcohol. Finally, plasma concentrations of NfL and BDNF and certain alcohol-related variables demonstrated to be robust factors for detecting cognitive decline in SUD patients with high precision (94%), which could be useful at preventive level to implement better therapeutic strategies with the aim of avoiding neurocognitive deterioration.

Limitations and future directions

The current study has limitations that future research should consider. Firstly, the study has a small sample size of dementia patients that also lacks a thorough phenotype diagnosis. Future studies should examine these effects in alcohol use patients comparing with particular neurologic groups (i.e., Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, etc.). Secondly, the study also has a small sample size of patients with cocaine and cannabis use disorders that worsen the evaluation of NfL and BNDF markers, so future research should extend the clinical sample to these diagnosis. Third, despite we adjusted age in our group comparisons, we did not consider age for correlations between NfL and BNDF with addiction-related variables. Since age influences these biomarkers, a broader sample, including a significative age span, or even better, longitudinal follow up studies should be done to clearly delimitate the impact of age. Finally, the study lacks a significant representation of the female population, which prevents the investigation of gender/sex differences in NfL and BDNF markers. This is a key issue that reflects the stigma of alcohol use disorder in women population that only demand treatment by health services at advanced stages of their disorder.

MATERIALS AND METHODS

Recruitment and screening of participants

The present study included 127 volunteer participants of Caucasian origin divided into three groups: 60 abstinent SUD patients (SUD group) in outpatient treatment, 27 patients from neurology outpatient settings (dementia group) and 40 control subjects (control group). SUD patients were recruited at the *Centro provincial de Drogodependencias* (Málaga, Spain). Among SUD patients who attended to outpatient treatment for addictions, 22 went for alcohol use as first complain, 33 went for cocaine use and 5 went for cannabis use. However, 36 SUD patients presented high comorbidity with other SUD's, specially between AUD and cocaine/cannabis use disorders. Dementia patients were collected at the Neurology Service of the *Hospital Regional Universitario de Málaga* (Málaga, Spain). The dementia group was composed of 9 patients with mild cognitive impairment, 7 patients with Alzheimer's disease, 6 patients with subjective complains, one patient with vascular dementia and one patient with stroke and 3 did not have specific diagnosis. Control participants were included from databases of healthy subjects of the *Biobanco Nacional de AND* (Valencia, Spain).

To be included in the present study, the SUD group had to meet the following inclusion criteria: people ≥18 years in the abstinence phase and willingness to participate by signing the informed consent. The exclusion criteria included: personal history of long-term inflammatory disease or cancer, language severe limitations, pregnant or breast-feeding women and infectious diseases such as Hepatitis C, Hepatitis B and HIV. The dementia group was used as a clinical control of our SUD sample as well as the healthy controls due to their high levels of plasma NfL are well known. Thus, the dementia group had to meet the following inclusion criteria: people ≥60 years under neurologic treatment with a Mini-Mental State Examination (MMSE) score <24 and willingness to participate by signing the informed consent. The exclusion criteria included: personal history of alcohol use during the last year using an Alcohol Use Disorder Identification Test (AUDIT) score >8, language severe limitations and infectious diseases such as Hepatitis C, Hepatitis B and HIV.

Regarding the control group, participants with history of substance abuse, psychiatric comorbid disorders, medic illness and cognitive impairment were also excluded.

Ethical statement

Written informed consents were obtained from each participant after a complete description of the study. All participants had the opportunity to discuss any questions or problems. The present study fits within the framework of projects promoted by the *Red de Trastornos Adictivos [RTA]* (Addictive Disorders Network), an entity financed by the Instituto de Salud Carlos III (ISCIII), belonging to the Ministerio de Ciencia e Innovación of Spain. The ethical aspects of the core project (Proteomics of Cocaine Addiction: Central and Peripheral Biomarkers of Addiction) were approved by the Ethics and Clinical Research Committee of the Regional University Hospital of Malaga in accordance with the Ethical Principles for Medical Research with Human Subjects adopted in the Declaration of Helsinki by the World Medical Association (64th General Assembly of the WMA, Fortaleza, Brazil, October 2013) and Recommendation No. R (97) 5 of the Committee of Ministers to the Member States on the protection of medical data (1997), and the Spanish law on data protection [Regulation (EU) 2016/679 of the European Parliament and of the Council of April 27, 2016 on the protection of natural persons with regard to the processing of personal data and the free circulation of such data, and repealing Directive 95/46 / EC (General Data Protection Regulation). All collected data received code numbers to maintain privacy and confidentiality.

Psychiatric and neuropsychological evaluation in the SUD group

The Spanish version of the PRISM (Psychiatric Research Interview for Substance and Mental Diseases) diagnostic interview was used for the evaluation of SUDs and other psychiatric disorders according to the criteria of the DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, 4th edition). The PRISM is a semi-structured interview with

good psychometric properties in the evaluation of substance use disorders and in the main comorbid psychiatric disorders related to the substance use population[59]. To give a one-dimensionality to the DSM-IV-TR criteria for abuse and dependence, we used the sum of the abuse and dependency criteria [1-11 criteria]. This criteria is in accordance with the DSM-5 diagnostic manual (See Araos et al., 2015)[60].

The neuropsychological evaluations for the SUD group were carried out individually and the duration of each session was approximately one hour. Data collection and evaluations were performed by psychologists trained in neuropsychological evaluation. We defined a value of -1 standard (Z) score as the cutoff for mild cognitive impairment and a value of -2 standard score as the cutoff for severe cognitive impairment as previous studies reported[61,62]**.** Moreover, Z scores were calculated taking into account the age and the educational level when the tests required. The neuropsychological battery was performed using different tests that have been demonstrated to be the most appropriated for detection of cognitive impairment in these patients[20] and are detailed below:

- Montreal Cognitive Assessment (MoCA) is a brief screening test that assesses general cognitive status[63]. MoCA evaluate multiple cognitive domains including attention, concentration, functions executive, memory, language, visuospatial skills, abstraction, calculation and orientation. The total score of the MoCA test is 30 points, and the administration time is 7-10 minutes, depending on the patient's condition. For obtaining the cut-off points to consider mild, moderate, and severe cognitive impairment, direct scores were adjusted for age and educational level[64]. This assessment has adequate internal consistency, the results are stable over time and presents good test-retest and inter-examiner reliability (Gallego et al., 2009).
- TAVEC is used to assess verbal episodic memory[65] . TAVEC structure is based on the Atkinson-Shiffin memory model[66]. Therefore, this test can provide information about coding or learning, short- and long-term storage, recognition, and the central

executive attention system. The test consists of learning a list A of 16 words that are divided into 4 semantic categories (4 words to each category): fruits, tools, clothing and spices. It also has a B-list of interference. This allows determining the "normality" of the subject by comparing with its age group.

- Trail-Making Test (TMT) was used to assess selective attention, working memory, impulsivity, focus and motor execution, speed of psychomotor processing and cognitive flexibility[67]. In addition, part B of the test assesses executive functions such as divided and alternate attention. In part B, the subject must connect and alternate between numbers and letters in ascending and alphabetical order. Direct scores were adjusted for age and educational level.
- Rey-Osterrieth complex figure test (ROCF) evaluate constructive and visuospatial capacity, perceptual organization and visual memory[68]. The test consists of copying a complex geometric figure. After a certain time of the phase of copy (an interval not exceeding 3 minutes), the subject is asked to reproduce the figure without having. Thus, a blank sheet is provided and the execution time of the test is controlled with a timer. The reproduction does not necessarily have to be exact, but that details and proportions must be considered.
- Digit span subtest (WAIS-IV) assesses verbal working memory, mental manipulation, flexibility cognitive and memory capacity[69]. This test consists of two tasks in which a 9-digit sequence is presented as maximum and the subject must repeat the digits in the same order (forward) and reverse order (backward). The duration of the test is around 10 minutes. Direct scores were adjusted for age.

Obtaining plasma samples

Blood samples were obtained in the morning after an 8-12 hour fast (before psychiatric interviews). Venous blood was extracted into 10 ml K2 EDTA tubes (BD, Franklin Lakes, NJ, USA) and immediately processed to obtain plasma. Blood samples are

centrifuged rum at 2200 x g for 15 min (4 ° C) and individually tested for infectious diseases using 3 commercial rapid tests for HIV, Hepatitis B and Hepatitis C (Strasbourg, Cedex, France). Finally, the plasma samples were individually aliquoted, recorded and stored at -80 °C until further analysis.

Multiplexed bead immunoassay

Plasma concentrations of BDNF were measured by using a human custom 7-ProcartaPlex bead immunoassay kit (Invitrogen, cat. no. PPX-07-MXH6ANW) in a Luminex xMAP® technology - MAGPIG system (ThermoFisher). Sensitivity was approximately 57 pg/ml, mean intra-assay variation (%CV replicates) was 12.1%[70]. The minimum detectable concentration value was attributed to missing values that were under the standard curve.

Serum neurofilaments

Light Chain Neurofilament (NFL) concentrations were determined using a digital enzyme immunoassay and the SIMOA HD1 Analyser platform, as described[70].

Statistical analysis

All data in the tables are expressed as numbers and percentage of subjects [N (%)] or means and standard deviations (SD). The significance of the differences in the qualitative variables was determined through Fisher's exact test (Chi-square) and Mann-Whitney U test's, respectively.

Multiple analysis of covariance (ANCOVA) was performed to indicate the relative effects of explanatory variables (i.e., lifetime of SUDs, cognitive impairment) on the plasma concentrations of NFLs and BDNF. Because we could not match the SUD group and dementia group for age, we controlled this variable entering as a covariate. *Post hoc* tests for multiple comparisons were performed using the Bonferroni correction test. The effect size was measured using partial eta squared (η_p^2). Correlation analyses were performed

using the Spearman's coefficient (rho). The normal distribution of the variables was assessed using Lilliefors corrected Kolmogorov-Smirnov test. As the NfL, BDNF and BDNF/NfL ratio variables of the study did not meet the assumption of normality, logarithmic transformations (10) were used to preserve parametric assumptions for positively skewed distributions and estimated marginal means [95% Confidence Interval (95% CI)]. Then, we used the antilogarithm of the concentrations to represent them in the figures.

To determine the variables that were able to discriminate between SUD patients with and without cognitive impairment, a binary logistic regression analysis was performed using Pearson's Chi-square (χ^2) test meeting the Hosmer-Lemeshow test. We assayed multicollinearity by examining Tolerance and Variance Inflation Factor (VIF). The cut off value for Tolerance was >0.10 and <10 for VIF. The variables included in the models were NfL, BDNF and alcohol-related variables.

We determine Z scores considering the mean and standard deviation provided by manuals of the neuropsychological tests and were adjusted for age and education when required. Thus, Z scores were calculated resting the direct score to the mean and dividing with the standard deviation.

Statistical analyzes were carried out using GraphPad Prism version 5.04 and IBM SPSS Statistic version 22 (IBM, Armonk, NY, USA). A value of $p < 0.05$ was considered statistically significant.

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Conclusiones parciales del Estudio 4:

- La concentración plasmática de NfL es mayor en el grupo TUS y el grupo de demencia que en el grupo control.
- Las concentraciones plasmáticas de BDNF fueron menores en el grupo TUS en comparación con el grupo control.
- Existe una correlación negativa entre NfL y BDNF en el grupo TUS y el grupo control, pero no en el grupo de demencia.
- La relación BDNF/NfL es mayor en el grupo TUS y el grupo de demencia que en el grupo control.
- Los niveles plasmáticos aumentados de NfL y niveles plasmáticos disminuidos de BDNF se observan en pacientes TUS con deterioro cognitivo moderado-grave en comparación con aquellos sin deterioro cognitivo.
- Los niveles plasmáticos de NfL se asocian con dominios de la función ejecutiva y la cognición de la memoria.
- Los cambios en las concentraciones plasmáticas de NfL y BDNF estuvieron mediados por el consumo crónico de alcohol, pero no por consumo de cocaína y cannabis a lo largo de la vida.
- Existen correlaciones positivas entre la edad de inicio de TUA, los años de duración del TUA y los NfL.
- Existen correlaciones positivas entre la edad de inicio de TUA, la gravedad del consumo de alcohol y el BDNF.
- Los niveles plasmáticos de NfL y BDNF no se ven afectados por los trastornos depresivos ni de ansiedad comórbidos.
- Las concentraciones plasmáticas de NfL y BDNF y las variables relacionadas con el alcohol son buenos predictores para discriminar entre pacientes con TUS con y sin deterioro cognitivo con alta precisión (94%).

4.5. Revisión

Reserva cognitiva y trastornos por uso de sustancias: una revisión actual de estudios preclínicos y clínicos

“Cognitive Reserve and Substance Use Disorders: A Current Review of Preclinical and Clinical Studies.”

COGNITIVE RESERVE AND SUBSTANCE USE DISORDERS: A CURRENT REVIEW OF PRECLINICAL AND CLINICAL STUDIES

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Keywords: Cognitive Reserve, Substance Use Disorder, Cognitive Impairment, Biomarkers.

Highlights

- CR is the capability of an individual to cope with a brain pathology through compensatory mechanisms.
- Subjects with SUDs show abnormalities in attention, memory and learning and executive functions.
- EE can be understood as a widespread intervention that enhances CR in rodents. However, a CR consensus in laboratory animals is still needed.
- CR has a protective role against substance use initiation, substance use patterns, drug-related cognitive impairment, and improves responses to treatment.
- Neurotrophines such as BDNF and 3-NT could be potential targets of CR.

ABSTRACT

Background: Cognitive reserve (CR) refers to an active coping process through pre-existing flexible and effective neural networks as a result of cognitive stimulation throughout life. Alternatively, addiction is a neurobiological disorder characterized by neurochemical dysregulation and neuroadaptations in brain areas involved in reinforcement, emotional regulation, learning, behavior inhibition and habit formation.

Objective: This review summarizes how CR can protect from normal aging processes to the appearance of neuropathological diseases. We also explain Environmental Enrichment (EE) paradigm as an effective intervention for rodents to imitate a healthy lifestyle in humans. However, although CR is a new concept highly demanded in research, little is known about its impact in the field of drug addictions.

Method: We performed a selected overview of clinical and preclinical studies about the role of CR and the EE condition in preventing drug use and its cognitive decline. In addition, we also discuss potential CR biomarkers that could be used to monitor subjects at risk of cognitive impairment or substance use onset.

Results: The scientific literature suggests that CR could act as a useful resilience-promoting factor in preventing uncontrollable drug use patterns and involvement and in reducing their deleterious impact on cognition. Additionally, we expose Brain-Derived Neurotrophic Factor (BDNF) and Neurotrophin-3 (NT-3) as potential biomarkers of CR.

Conclusion: both preclinical and clinical models support CR as a protective factor for drug addiction and drug-induced neurocognitive impairment. Finally, although we are still far from establishing a specific biomolecule as a CR biomarker, evidence indicates BDNF as a promising candidate.

Keywords: Cognitive reserve; Addiction; Substance Use Disorders, Environmental Enrichment, Brain-Derived Neurotrophic Factor

1. Introduction

The construct of 'reserve' has been proposed to explain the discrepancy between degree of brain pathology and cognitive performance expected across individuals[1,2]. This phenomenon is thought to be determinant explaining how patients with similar brain damage (onset, extension, location) show different cognitive profiles. The mechanism by which the 'reserve' may protect from cognitive decline include 'brain reserve', which refers to a passive coping process through brain structures (i.e., brain size, cortical thickness, white and gray matter integrity, dendritic length and density[3,4]), and 'Cognitive Reserve' (CR) which refers to an actively coping process via preexisting flexible and effective neuronal networks as a result of cognitive stimulation throughout life[5]. Evidence support that CR seems to be the result of premorbid IQ, years of education, occupation attainment, leisure activities, physical activity, and social interactions[6,7]. It is noteworthy that years of education is one of the most studied CR proxies since the other factors can be inferred from this measure[8]. However, brain reserve and CR are not mutually exclusive since exposure to cognitive stimulation throughout life experiences can create structural and functional changes in the brain.

Therefore, strong evidence is that a high CR can protect in a wide range from normal aging processes[9] to the appearance of neuropathological conditions, such as mild cognitive impairment[10], Alzheimer's disease[11,12], Parkinson's[13,14], multiple sclerosis[15], brain damage related to trauma, stroke or tumor[16, 6]; and psychiatric disorders such as depression, bipolarity, anxiety or psychosis[17-20]. However, despite that an elevated CR softens clinical manifestations and slows progress from subjective cognitive decline to mild cognitive impairment (MCI), it seems to be related to a faster cognitive decline toward dementia after onset of MCI symptomatology[10, 21]. This probably evidence the protective role of CR is limited to early stages of brain injury that retains the disease but is not able to deal with brain damage in later stages.

According to potential brain areas related to CR, findings of a systematic review in fMRI studies suggest that medial temporal lobe regions are associated with high CR in young adults showing to be resistant to neurodegeneration. In contrast, frontal regions were recruited to compensate for regions that are affected in older adults and MCI/Alzheimer's disease (AD) patients with high CR[22]. In addition, in a meta-analysis of fMRI studies, CR was related to medial-lateral frontal regions as anterior cingulate cortex, dorsolateral prefrontal cortex, and precuneus in older adults. Instead, only functional activation in the anterior cingulate cortex was associated with CR in MCI/AD patients[12]. Considering this, both a low activation of medial temporal lobe areas and the increase in compensatory frontal networks reflect the role of CR at first stages. Afterward, activation of specific frontal areas shows the last blows of CR as a result of neurodegeneration. Accordingly, CR engages some domains of executive functions as working memory, verbal fluency and interference, but not to emotion perception, processing speed or motor performance[23]. Unlike preclinical studies, benefits afforded by CR may not relate to social cognition[24, 25].

On the other hand, drug addiction is a neurobiological disorder in which, after prolonged use of drugs of abuse, neurochemical dysregulation and neuroplastic changes (neuroadaptations) occur in brain areas involved in reinforcement, emotional regulation, learning, behavior inhibition and habit formation. These neuroadaptations are considered responsible for the etiology and long-term maintenance of the addictive disorder -in which the individual loses control over the intake of the substance- and for the clinical symptoms that accompany addiction[26]. Importantly, certain addiction-related areas may overlap with those supporting CR. For example, the frontal cortex which is responsible for executive function and behavioral control including inhibition of drug-seeking and taking habits. In fact, the construct of CR has recently been established in the field of addiction, suggesting a preventive role in the onset of drug use, less severity of consumption, fewer cognitive impairment, and better response to treatment[2]

2. The importance of cognition in drug addiction

Cognitive processes play an important role in drug addiction. In fact, authors have identified a cognitive phenotype that would make the individual more prone to initiate and maintain drug use. Specifically, as evidenced by both clinical and preclinical research, the addiction-vulnerable cognitive profile seems to involve mainly frontal symptoms indicative to 'loss of control' over behavior, such as increased impulsivity and a reduced ability to inhibit inappropriate responses[27, 28]. Other cognitive-related traits frequently associated to drug use or addiction are disadvantageous decision making and risk-taking[29], increased sensation-seeking and novelty response[30] and a reduced 'insight'[31] (i.e., interoception or self-awareness).

Furthermore, exposure to addictive drugs triggers neurocognitive impairment through aberrant neuroplasticity that widely compromise the integrity of learning-related brain regions[32-34] (see Figure 1) (Castilla-Ortega et al. 2016; et al. 2001; Sampedro-Piquero et al. 2018). In preclinical studies, naïve rodents that are repeatedly administered drugs -or self-administer drugs- show deficits in working and reference memory that may persist even after weeks or months of drug abstinence[35-39]. In addition to this memory decline, rodents exposed to drugs may also exacerbate those cognitive-like vulnerability traits associated with uncontrollable drug use, such as is the case of impulsivity[40, 41].

In agreement with preclinical evidence, persons with substance use disorders (SUDs) show cognitive impairment in a wide range of domains [including attention, psychomotor functions, executive functions (behavior inhibition, abstract reasoning, planning, decision making), working memory, short- and long-term declarative memories (verbal, spatial) compared to non-using controls[34,42,43]]. A challenge in clinical studies is to elucidate which cognitive features were previous or a consequence to the drug. Overall, it seems that the cognitive deficits associated to SUDs are progressively ameliorated by protracted drug abstinence, often finding a significant improvement in all or in several

cognitive functions within one year without using drugs[44-47]. This suggests that cognitive dysfunction in persons with SUDs is, at least in part, secondary to substance abuse. Nevertheless, there are also cases of long-lasting, and apparently irreversible cognitive impairment[48-50].

Therefore, it is important to note that cognitive decline in persons with SUDs may involve different degrees of persistence and severity. In addition to the duration of drug abstinence, drug use patterns seem to act as modulators of the cognitive impairment associated with SUDs, explaining the discrepancies among studies and individuals. In this regard, it has been suggested that the drug-induced cognitive decline is dose-dependent, in a way that more severe neurocognitive impairment may be found in heavier drug users, regarding more quantities of drug consumed and/or more years exposed to the drug[47,52,53]. There is also an influence of the type of drug used. For example, compared to other drug types, alcohol abuse seems especially associated to more profound and lasting cognitive deficits –even leading to dementia and other neurocognitive syndromes such as Wernicke-Korsakoff, probably due to its potent neurotoxic actions-[46,48-50]. Polysubstance abuse (i.e., dependence on at least two drugs) has also been associated with a long-lasting cognitive dysfunction with minimal recovery over a 1-year period[42] though, in certain populations, it is unclear whether abusing additional drugs may add severity to cognitive symptoms[54]. Another relevant factor is the age of drug use onset, since drugs acting in periods of the life span when the brain is immature and not yet fully developed (i.e., adolescence) may yield more lasting consequences[47,49,55].

In any case, the degree of cognitive impairment has been revealed as a reliable predictor of a reduced adherence to the addiction treatment and increased risk to relapse in drug use[34,56]. Impaired executive functioning could notably contribute to drug addiction maintenance, for example by impeding refraining from drug use or by hindering abstract

reasoning processes that may lead to disadvantageous planning, maladaptive decisions, reduced comprehension of treatment programs or weaker motivation toward change.

Another avenue for cognitive impairment to impede recovery relies on memory processing. On the one hand, extinguishing previous memories for drug-related experiences (i.e., drug-stimuli associations) requires substantial cognitive engagement[57]. Extinction is not a mere memory decay but an active learning process to assimilate that one stimulus no longer predicts the other. Extinguishing the memories associated to the drug is highly convenient, because the presence of drug-associated stimuli (i.e., places, people, objects, songs, even thoughts or feelings) trigger drug craving and uncontrollable (habitual) responses of drug seeking and intake, ultimately leading to relapse[26]. In addition to impair the extinction of drug-associated memories, a defective learning and memory could potentially prevent acquisition and consolidation of new adaptive information, such as the instructions for addiction therapy follow-up, or memories for significant non-drug related experiences. It is important to note that cognitive-behavioral therapies for addiction require a set of both executive and memory skills that, ironically, may overlap with those cognitive functions compromised by drugs[58].

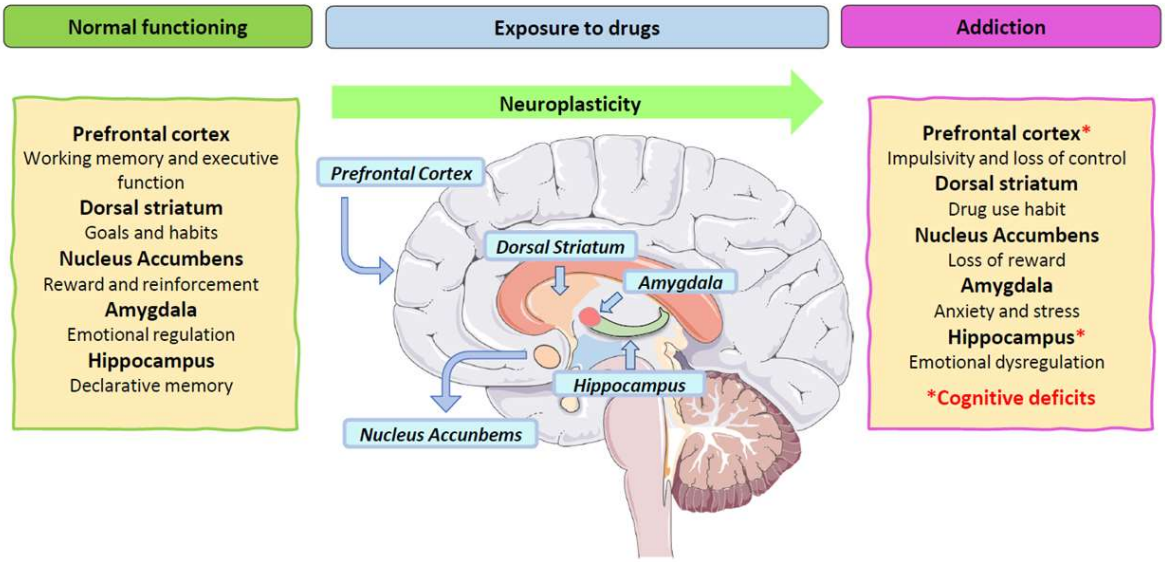

Figure 1. Neurobiological bases of addiction. Drugs, including alcohol, produce neuroadaptations in the brain, which contribute to the generation and maintenance of the addictive disorder as well as the symptoms accompanying clinicians.

3. Cognitive reserve in animal models of addiction

Despite how well-established CR is in humans, it is still necessary to reach a consensus on which are the techniques of choice to potentiate CR in laboratory animals. Nevertheless, the widely used Environmental enrichment (EE) paradigm (see Figure 2) has been proposed as an effective intervention for rodents that mirrors a cognitively healthy lifestyle in humans[59]. In experimental settings, EE typically includes a larger home-cage that is full of objects –i.e., toys, ladders, tunnels, etc.- to experience novel stimuli, a larger group of companions to enhance social interaction, running wheels for promoting voluntary physical exercise, or even novel odors and foods[59]. This setting contrasts with the standard or 'control' condition that usually entails a rather impoverished environment where mice or rats are housed in small groups or isolated, in a home-cage filled only with few elements required for animals' welfare (i.e., bedding, and nesting material).

While the EE protocols may widely vary among laboratories -mainly regarding their duration and the stimuli involved-, literature strongly suggests that EE provides a 'reserve' in rodents that allows to compensate cognition in the case of insult. Therefore, EE would meet the requirements to be considered as a preclinical intervention to enhance CR[59,60]. Firstly, EE is highly effective to trigger experience-dependent plasticity in the whole brain (i.e., 'brain reserve'), which includes the limbic regions that oversee cognitive processes. For example, EE widely sculpts the circuitry of the prefrontal cortex and the hippocampus as it induces molecular, electrophysiological and morphological changes, such as augmented synaptic plasticity and neurotrophic factor levels[59,61,65]. In the rodent hippocampus, EE even increases the pool of adult-born neurons, by stimulating both new neurons' birth and their long-term survival[66]. Secondly, rodents exposed to EE enhance cognitive performance in both prefrontal and hippocampal-dependent domains. This has

been elucidated by a variety of memory tasks such as long-term novel object recognition, spatial navigation or associative memory extinction[67-70]. Notably, the EE-housed rodents performing cognitive tasks display a different pattern of brain activation compared to non-enriched controls[69,70]. This shows that EE stimulates alternative brain networks that are relevant and beneficial for cognitive processes. Finally, remarkable evidence is that EE can protect from the cognitive impairment induced by a wide variety of detrimental conditions that compromise brain neuroplasticity, such as brain injury, chronic stress, aging or dementia-like models[71-75].

In this regard, it is known that EE modulates the brain consequences of drugs, since the neurochemical regulation, neurotrophic factors levels and brain functional activation patterns are different when the drugs are administered to EE-housed animals[76-80], and EE is even able to protect from drug-associated neurotoxicity and pro-inflammatory response[76,81]. Accordingly, EE-housed animals exposed to drugs perform working memory tasks similarly to drug naïve controls[78,82,83]. This has been evidenced for different classes of drugs such as ethanol, nicotine and methamphetamine, in animals that were exposed to EE either from their birth or in their young adulthood[78,82,83]. However, although these preliminary studies show promising results, their small number prevents solid conclusions from being drawn.

While there is scarce research on the effects of EE to prevent the neurocognitive consequences of drugs, the effect of previous EE exposure on drug-induced psychomotor activation, as well as on the engagement in drug seeking and taking responses, have been thoroughly investigated[34,65,84]. Considering that EE widely sculpts brain neuroplasticity, it is not surprising that EE-exposed animals show different locomotor activity after drug administration, but there is not yet a consensus on whether such effect may be decreased or enhanced by EE[34,65]. The results are also ambiguous regarding the drug-induced conditioned place preference (CPP) paradigm, which assesses drug reward by studying preference for one context (i.e., a maze compartment) that is paired with the effects of a drug, in

comparison with a neutral context paired with saline administration. In the CPP task, animals that are conditioned after or while experiencing EE, may display either unaltered, increased or reduced CPP response[34,65]. A possible explanation is that the associative memory capacity plays an important role for learning the drug-context associations that are required for CPP expression, so the cognition-enhancing effects of EE could obscure its potential outcome in reducing drug motivation and reward in a CPP setting. Considering this, the drug self-administration paradigm is the preferred preclinical model to study motivation for drugs, since it allows a direct assessment of drug-taking by allowing the animal to press a lever that results in a drug infusion. Conversely, literature has been quite consistent in showing that EE-reared animals would reduce self-administration of a variety of drug types including alcohol, nicotine or psychostimulants[34,65,84]. Because of this, EE is currently considered as an intervention to prevent addiction-like behaviors in rodents[34,65,84]. Nevertheless, a notable limitation is that few experiments have used female animals or have focused on gender differences. It has been reported that the effect of EE to reduce amphetamine self-administration is similar for both sexes[85] but this is not clear for other drugs[86].

The mechanisms by which EE may protect from engaging in drug intake are numerous and they probably involve an interplay of emotional, motivational and cognitive components[65]. Thereby, EE usually improves emotional regulation, reducing anxiety and depression-like responses and increasing resilience to stress[65,87-89], which is not surprising considering that the same limbic regions implicated in cognition are also a key locus for emotion[90]. In fact, a recent study reveals that EE prevents the detrimental effects of social stress on ethanol intake in vulnerable animals[82]. On a different note, EE widely sculpts the reward system by providing alternate rewarding experiences (exercise, social interaction, novel stimuli), that could diminish the motivational value of drugs, since non-enriched animals seem more sensitive for rewards[65,91].

Another possibility, which is more in line with the concept of CR, is that EE could enhance a set of cognitive skills that provide a resilient phenotype for drugs. In this way, EE could reduce drug seeking by strengthening the prefrontal-dependent functions of behavioral control and behavioral inhibition[92]. While EE certainly modulates impulsivity, the direction of the change is still unclear. EE-reared animals may show reduced impulsive and risky choices[93-96], though there are a number of studies that found that EE increased impulsivity instead[91,97,98]. An impulsive-like behavior such as novelty seeking is typically attenuated in enriched animals that would show less preference for novelty, reduced locomotor activity and faster habituation in a novel environment[93,97,99-101]. In any case, the apparent actions of EE to modulate impulsive-like responses may be explained by increased behavioral control and/or by a reduced reward incentive. For example, EE decreases ability to discriminate between small and larger food rewards –used in impulsive choice tasks[91]- and reduces the incentive value for visual novelty[102]. In addition, EE may also modulate anxious phenotypes that are often associated with impulsive responses[103]. Therefore, both motivational (i.e., reward incentive) and emotional (i.e., anxiety or fear) traits could affect interest in novelty, risky decision-making, or impulsive-like behavior.

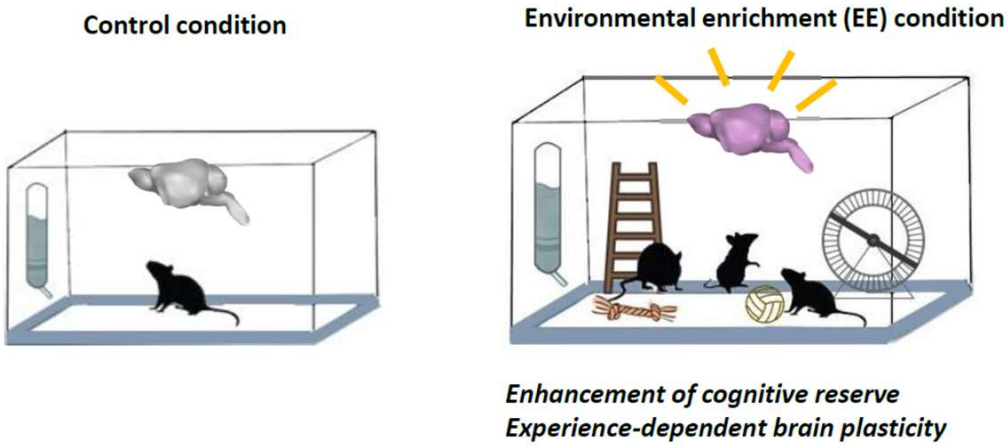

Figure 2: The Environmental Enrichment (EE) paradigm proposed as an effective intervention for rodents. EE typically includes a larger home cage that is filled with objects, to experience novel stimuli, a larger group of companions to enhance social interaction, running wheels to promote voluntary physical exercise, or even novel smells and foods. This configuration contrasts with the standard or

'control' condition that generally implies an impoverished environment where rodents are housed in small groups or isolated, in a domestic cage filled with only a few items necessary for the well-being of the animals.

4. Cognitive reserve in clinical studies of addiction

While CR is a new highly demanded concept in cognitive research, little is known about its impact on the field of substance addictions. Current evidence suggests that CR in SUDs contributes to improved neuropsychological performance and it has been related with less indicators of neurocognitive disorders[104]. Also, an elevated CR, derived mainly from educational level, has been related to better prefrontal function in daily life in patients with SUDs[8]. Specifically, global cognitive integrity has been mostly related with years of education and with leisure activities to a lesser extent, whereas work-related activities have been associated with sequencing and visuospatial inhibitory response in polysubstance patients[105]. Regarding the type of drug used, scientific literature has particularly focused on how CR is able to prevent the adverse effects of alcohol and cocaine overconsumption on cognitive function; whereas opiates, sedatives, and stimulant drugs are still completely unexplored.

Focusing on alcohol abuse, a longitudinal study pointed that an educational level lower than high school and a low job occupation is associated with an increased risk of dementia in alcohol patients[106]. Middle-aged adults also show alterations in psychomotor speed, attention, and reasoning skills when they have low occupational and educational attainments[107]. Furthermore, consumption of high amounts of alcohol has been associated with poorer cognitive performance in patients with low socioeconomic status[107]. It is important to note that cognitive dysfunction in alcohol use patients may not be affected by psychiatric affective comorbidity[108]. Even individuals under an acute intoxication with a poor CR may present more difficulties when they make complex decisions such as having sex without protection[109]. Moreover, CR can act as a protective factor in presence of the neuropsychological VIH-related impairments in intravenous drug patients[110]. Regarding

cocaine abuse, a high premorbid Intelligent Quotient (IQ) seems to be a protective factor against cognitive deterioration as in the domains of verbal learning, declarative memory and working memory domains[111]. Similarly, it has been observed that decreased processing speed and attentional impairment are mediated by CR in cocaine patients[25]. Finally, it has been described that psychotic patients who use cannabis obtained better cognitive performance (verbal learning, working memory span, planification) than those who do not use and these differences were explained by premorbid IQ between groups[112].

In addition, the extent of CR not only influences cognitive performance in SUDs patients but also affects the addiction-related variables. In this regard, a recent review has described the preventive role of CR on drug use onset, development of addiction, severity of substance-related problems and better treatment outcomes[2]. Thus, evidence suggests that educational achievement is determinant in abuse and dependence progression on illicit substances in adolescents[113]. Young people who dropped out of high school or college have a higher risk of developing alcohol abuse in adult life compared to those who completed these academic achievements[114]. Similarly, findings revealed that cocaine use patterns are strongly related with lower socioeconomic status and lower parental education as well as scholar absence and low level of leisure reading[115]. Furthermore, the most common negative consequences expressed by the students who were substance users were absent from classes, memory problems and poor academic performance[116]. Regarding adulthood, a study informed that a high CR led to lesser severity of addictive process and a longer duration of abstinence in SUDs patients[8]. Unstable patterns of alcohol consumption have been particularly related to poorer cognition in the low socioeconomic group operated by educational and occupational achievements[107]. Also, our results indicated that lower educational levels were accompanied by earlier onset of alcohol consumption, earlier development of alcohol dependence and impaired frontal cognitive function. Several studies have found that unemployment was associated with the increased risk of alcohol

consumption at follow-up and they are also more likely to be smokers and use illicit and prescribed drugs as well as develop SUDs[117,118]. In EEUU, gender discrimination has been consistently associated with the use of illicit substances and SUDs among women showing a position of greater risk those with an educational level below secondary[119]. Similarly, our group has recently found that the educational level can play a protective or a risk role in the onset, development, and progression of cocaine use disorders[120]. In addition, other researchers have observed that an elevated CR is associated with lower levels of cocaine craving[25].

Nevertheless, distinguishing whether the development of some SUDs is a cause or consequence of poor educational and occupational attainments is one of the most complex issues on this matter. However, both academic dropout and unemployment could predispose to the onset of drug use since students would be deprived of protection offered by the educational system and years of schooling or employment in case of adults. Similarly, it has been found that healthy leisure activities such as physical exercise, reading books and going to the cinema have been associated with decreased risk of substance use. In contrast, sedentary leisure activities reflected by playing video games and watching TV or going out with friends to have a drink showed the opposite effect, observing drug use among adolescents and young people[121,122]. Also, social support consisting of good family relationships has been proved to be a protective factor against onset of drug use[123,124]. Therefore, the lack of cognitive stimulation derived from leaving these activities through life might interfere with brain plasticity increasing posterior cognitive vulnerability[125,126].

5. Potential biomarkers of cognitive reserve in substance addiction

Biological markers or biomarkers are measurable biological, chemical, physiological, and anatomical substances that can be related to a normal biological state, the development of a specific disease or organic responses to exposure to certain environmental variables (for example, medical or psychological treatment)[127]. Some investigations have

analyzed the presence of biomarkers to elucidate which factors related to CR dampen the cognitive deterioration derived from normal aging and several neuropathologies[1].

On this line, exposure to an EE produces alterations in gene and protein expression of patterns[128] and regulate biochemical processes related to growth factors, neurotransmitters[129], hormones[130-132] and the immune system[133]. Thus, studies which evaluated the effects of the EE paradigm, found that factors related to CR favor neurogenesis, neuroplasticity, neuroprotection, synaptogenesis, angiogenesis and gliogenesis through increased expression of certain proteins in rodents[134-136]. Among them, a family of proteins namely neurotrophic factors or neurotrophins are involved in neuronal survival, growth, differentiation, and protection and are expressed in areas essential for memory, learning, and executive functions such as the hippocampus and prefrontal cortex[137]. Specifically, brain-derived neurotrophic factor (BDNF) has been extensively related to CR and EE mechanisms[135,139].

Regarding preclinical research, higher levels of BDNF in the hippocampus and the medial and orbitofrontal prefrontal cortex has been significantly associated with better cognitive performance in memory and learning tasks as well as higher behavioral performance in cage-reared rats environmentally enriched compared to those raised in conventional cages[139,140]. Moreover, EE increased BDNF expression and cognitive performance as well as reduced behavioral deficits secondary to brain damage in ischemic stroke in rats[141]. Similar investigations linked the increase in BDNF levels with the mitigating effects of EE in cognitive deterioration in rodent models with accelerated senescence[136], with epilepsy[139] and of neurodegenerative diseases such as Alzheimer's[142], Parkinson's[143] and Huntington's disease[144]. Furthermore, exposure to EE significantly increases the transcription levels of the BDNF receptors tyrosine kinase (TrkB) in the ventral hippocampus of rats[139]. In addition, this study reflected higher transcription levels of

erythropoietin (Epo), a neuroprotective hormone that promotes the expression of BDNF in the cerebral cortex[145], as well as its receptor (EpoR) in the ventral and dorsal hippocampus.

According to preclinical studies, BDNF might be a potential biomarker to describe the neurobiological bases of CR in humans[138]. Physical activity, social interactions, cognitive stimulation, high educational level, and enriched environment have been associated with increases in BDNF levels and lower risk of dementia[146-149]. Conversely, it is known that the Val66Met polymorphism inhibits the release of mature BDNF, disrupts cognitive functioning[150-152], and interferes in the protective effect of CR on executive functions[153]. Regardless of this, there is an emerging line that supports the role of CR and the BDNF/TrkB signaling pathway as compensatory responses that delay symptoms in the early stage of Alzheimer's disease, while in more advanced stages they would not be sufficient to prevent neurodegeneration[10,21,154,155]. Additionally, numerous studies have found that physical exercise increases circulant concentrations of BDNF and improves cognitive function in age related mild cognitive impairment and schizophrenia[156,157,159]. Heisz et al suggested that healthy young adults with high response to exercise may benefit from increases in BDNF and insulin-like growth factor I (IGF-1) neurotrophic factors compared to those with a low response[147].

However, BDNF might not be the only neurotrophin involved in the cognitive enhancement induced by EE. In this regard, Fares et al., (2013) identified an increase in IGF-1 in the hippocampus of rats housed in enriched cages[139]. Similarly, other studies show increased nerve growth factors (NGF) and neurotrophin-3 (NT-3)[158-159]. Moreover, several studies have demonstrated that EE training was associated with higher levels of other indicators of neuronal proliferation, growth, and plasticity, such as vascular endothelial growth factor (VEGF)[139,160], fibroblast growth factor-2 (FGF-2)[161], pleiotrophin protein (PTN), PSD-95 protein, the NeuN protein and the Ki67 protein[136]. In addition, the scientific

literature supports the modulatory role of EE in histone proteins and DNA methylation, an essential epigenetic process that affects the formation and consolidation of memory[136,162].

On the other hand, identification of potential biomarkers is an important question that would allow evaluating the degree of CR and vulnerability of an individual to cognitive deterioration in populations with SUDs, forming the basis of different treatments, and being used as an indicator of the efficacy of such treatments[163]. However, the relationship between biomarkers of CR in SUDs has not yet been investigated. Previously, our team has found decreases in plasma concentrations of BDNF, NT-3 and IGF-1 in patients with alcohol use disorder, as well as an association between frontal functions and circulating BDNF in this population[164, 165]. Moreover, we have recently found that plasma concentration of NT-3 is higher in alcohol abstinent patients with a low education level compared to those with a high education level and could discriminate between them with a high accuracy. In addition, we also reported that educational level, NT-3 and BDNF might be compensatory mechanisms for brain damage in the early stage of alcohol use disorder, but not in later phases of the disease[166]. However, there are several altered biomolecules such as pro-inflammatory mediators[167-173], oxidative stress[174, 175] and lipids[176-179]in drug addiction (cytokines, chemokines, endocannabinoid;) that could serve as potential biological markers of CR and this relationship is completely unknown.

Conclusion and future directions

In summary, the relationship between cognition and SUDs is bidirectional: certain cognitive traits promote drug use, while drugs' neurobiological actions generate cognitive symptoms that would further exacerbate the SUDs and drug consumption, forming a 'vicious cycle'. Therefore, CR can be a promising resilient-promoting factor for lessening the deleterious impact of the drug on cognition and for preventing engagement in drug use (i.e., relevance for addiction severity and treatment) and uncontrollable drug use patterns (i.e., relevance for addiction prevention). Thus, cognitive stimulation for SUDs patients

with cognitive impairment could lead to improved cognitive abilities that in turn allow a better response to drug treatment and more opportunities of success[2,180]. Moreover, we should focus on early care measures in anticipation of future needs since school dropout and drug consumption lead to the impairment of cognitive functions and less cognitive reserve in adult life. However, due to the fact that many factors contribute to explaining CR, it is difficult to elucidate what each variable weighs on the benefits in the prevention of drug dependence, so future research is needed.

On the other hand, EE can be understood as a widespread intervention that enhances CR in rodents, which may protect from drugs' deleterious effects on cognition as well as from engaging in drug-taking responses. Thus, it is needed to expand the preclinical evidence on EE as a preventive strategy to understand the role of CR as a protective factor for drug addiction and drug-induced neurocognitive impairment. Therefore, it would be very valuable to seek complementary evidence by applying alternative interventions to enhance CR in rodents. Additionally, the scientific literature about CR lacks exploring gender differences in both preclinical and clinical studies.

Finally, the identification of potential biomarkers is an important question that would allow evaluating the degree of CR and vulnerability of an individual to cognitive deterioration. However, we are still far from establishing a specific biomolecule as a CR biomarker since we can only refer with the term biomarker to biomolecules robust enough to indicate the state of this neurological condition. Thus, a new line of research could be opened on whether CR can compensate for cognitive dysfunction derived from excessive substance use and which biomolecules are involved in this neuroprotective process.

Conflict of interest

None

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Contributors

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5. Discusión

Los estudios realizados en la presente Tesis doctoral demuestran claramente la relevancia de los trastornos cognitivos asociados al consumo de alcohol, así como la existencia de biomarcadores diagnósticos, pronósticos, y posiblemente de respuesta terapéutica, que permitirían identificar tempranamente el inicio del deterioro y posiblemente desarrollar intervenciones preventivas destinadas a mitigar el mismo y prevenir el futuro desarrollo de demencias.

5.1. Hallazgos principales.

Aunque se han descrito ampliamente los déficits cognitivos en personas con TUA, sigue habiendo mucho desacuerdo sobre la etiología y la cronicidad de éste. Mientras que algunos estudios apuntan que durante la abstinencia al alcohol se recuperan de forma total o parcial las funciones cognitivas, otros estudios señalan que estos déficits no solo son de carácter crónico, sino que podrían desencadenar una demencia de inicio temprano (Sabia et al., 2011; Schwarzinger et al., 2018b). No obstante, estas discrepancias se deben a que no se ha estudiado de forma correcta la influencia de las variables asociadas con el consumo problemático de alcohol sobre la cognición, como el inicio del consumo, el desarrollo del TUA, la longitud de la abstinencia actual y la duración del TUA a lo largo de la vida. Por otro lado, a pesar de que se existe una extensa información sobre la relación entre quimioquinas, factores de crecimiento y el consumo problemático de alcohol, todavía se desconoce el impacto que estas alteraciones pueden ocasionar en la cognición de estos pacientes. De este modo, la ruptura del desequilibrio funcional entre estas biomoléculas producido por el consumo problemático de alcohol podría subyacer a los mecanismos que explican la etiología y el desarrollo del deterioro cognitivo en esta población. Afortunadamente, en la actualidad, los efectos neurodegenerativos del alcohol se pueden determinar a través de la cuantificación de NfLs en sangre gracias a los nuevos avances en la tecnología de medición de molécula única (SIMOA) que hemos implementado en la

presente Tesis Doctoral. Es importante mencionar, que la presente tesis es la primera en describir las concentraciones plasmáticas de NfLs en pacientes con TUA. Además, a pesar de que el deterioro cognitivo y los factores neurotróficos han sido ampliamente descritos en la literatura científica, nunca se han relacionado de forma integrada con la reserva cognitiva y el nivel educativo; y aún menos en el campo de las adicciones. Por lo tanto, la gran novedad del presente estudio, y eje de la investigación de la presente tesis, se basa en la interrelación entre estos factores, tal y como se presenta en la Figura 21.

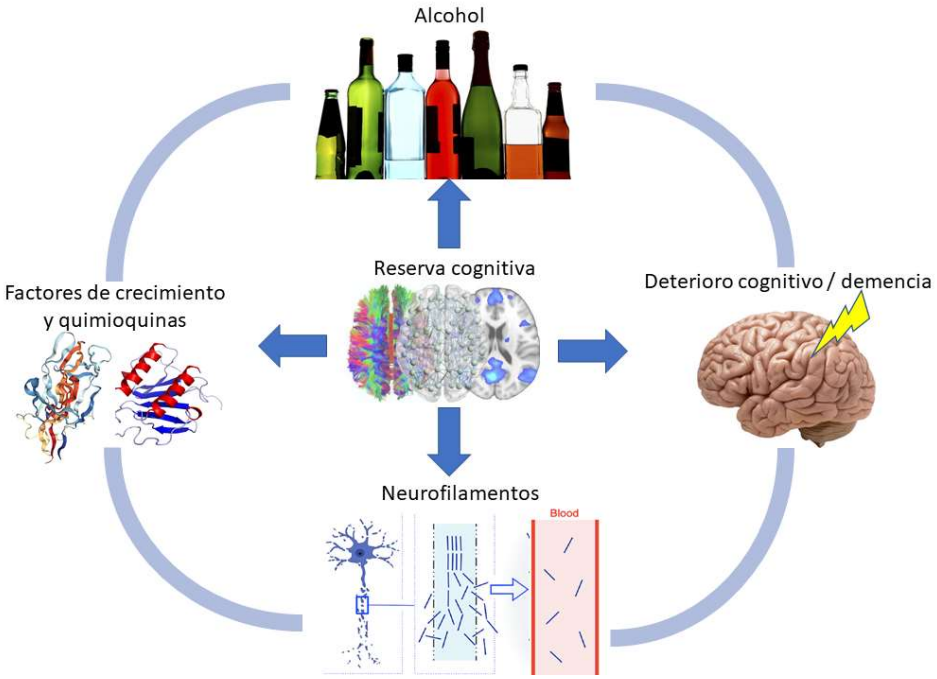

Figura 21. Diagrama resumen del eje de la investigación de la presente tesis doctoral.

En base a los resultados globales, podemos decir que mientras que la reserva cognitiva y ciertos parámetros de consumo (retrasar la edad de inicio, disminuir la intensidad) juegan un papel protector, la pérdida de los niveles tróficos de BDNF, y el aumento de algunas quimioquinas y del VEGFA juegan un papel opuesto, facilitando la aparición del deterioro cognitivo. Además, hemos podido identificar que este deterioro cognitivo tiene una base de lesión celular, gracias a la posibilidad de medir los neurofilamentos en sangre, que son para la neurología de hoy lo que en su día fueron las troponinas cardíacas: marcadores reales de daño celular (cardiomiocitos para las troponinas

y NfLs para el daño neuronal) (Thebault S, Booth RA, 2020). A continuación, discutimos estos hallazgos.

5.2. Deterioro cognitivo, alcohol y trastornos comórbidos.

Prevalencia y dominios cognitivos afectados:

Los estudios clínicos de la presente tesis arrojaron una prevalencia elevada de algún grado de deterioro de las funciones cognitivas en la población TUA que se situó en un 75% según la prueba MoCA. En concreto, un 55% de los pacientes TUA mostraron déficit cognitivos frontales: entre el 31-48% de los pacientes parecían tener signos de deterioro cognitivo similares al déficit frontosubcortical, mientras que entre el 23-26% tenían signos de deterioro similares a demencia frontosubcortical conforme con el manual del instrumento FAB. Por otro lado, el 36% de los pacientes TUA mostraron algún déficit subjetivo de memoria: el 19% presentaba problemas leves que tienen poco impacto en la vida diaria, mientras que el 17,2% reportó síntomas graves de deterioro mnésico con un impacto significativo en la vida diaria como se indica en el manual del MFE. Además, estos pacientes manifestaron déficits leves en el bucle fonológico, así como en el aprendizaje y memoria verbal a corto y largo plazo (DD, TAVEC). Además, manifestaron déficits graves relacionados con el deterioro de la atención ejecutiva y la flexibilidad mental (TMT B). Aunque no fue una diferencia significativa, también mostraron indicios de problemas leves en visoconstrucción (FCRO).

Reversibilidad de la función cognitiva:

Nuestros resultados indican que los pacientes TUA con deterioro cognitivo tenían más probabilidades de haber comenzado a consumir alcohol de forma temprana y de haber tenido un TUA de mayor duración a lo largo de la vida. Por tanto, son pacientes que se encuentran en un estadio más avanzado del trastorno y tienen la posibilidad añadida de sufrir una complicación hepática. En cambio, los pacientes sin deterioro cognitivo tenían un mayor número de criterios de gravedad de la adicción y una menor duración de la

abstinencia en el momento de la evaluación, lo que indica que se trata de pacientes que luchan activamente contra el trastorno. Estos hallazgos sugieren que el deterioro cognitivo no está asociado con la cantidad de alcohol consumido ni con la duración de la abstinencia sino con el inicio del consumo y la duración del TUA a lo largo de la vida, lo que concuerda, como veremos más adelante, con la idea de que la agresión producida por el alcohol de modo sostenido pueda generar una lesión a nivel axonal, liberando neurofilamentos a medida que se dañan las neuronas. Además, este resultado podría indicar un daño permanente en las capacidades cognitivas de los pacientes que consumen alcohol al no mejorar durante la abstinencia como indican otros estudios.

Deterioro cognitivo y comorbilidad psiquiátrica:

A pesar de que algunos estudios exponen que los déficits cognitivos encontrados en pacientes con adicciones pueden exacerbarse por un trastorno psiquiátrico comórbido (Lee et al., 2015), las diferencias en la función cognitiva encontradas en los estudios no se debieron a la comorbilidad psiquiátrica por otro trastorno depresivo o ansioso, que son los más prevalentes en esta población. Además, el hecho de que no se encuentren asociaciones significativas entre factores neurotróficos, NfLs y comorbilidad psiquiátrica, como se explicará más adelante, apoya aún más la viabilidad de este resultado.

Deterioro cognitivo y tipo de TUS:

Observamos una mayor prevalencia de deterioro cognitivo frontal en pacientes que han tenido un TUA a lo largo de la vida por sí solo, o acompañado de la adicción a otras sustancias como la cocaína o el cannabis (Figura 22). En concordancia, un estudio meta analítico sugiere que la evidencia actual no respalda la opinión de que el consumo crónico de cocaína se asocie con amplios déficits cognitivos, a pesar de que se han encontrado diferencias cerebrales y metabólicas en estos pacientes (Frazer et al., 2018). Aunque algunos estudios sugieren que el consumo prolongado de cocaína provoca deterioro en algunos dominios cognitivos, estos podrían deberse al consumo concomitante de alcohol

(Blanco-Presas et al., 2018). Del mismo modo, se necesita más investigación para determinar si existen alteraciones cognitivas asociadas con el consumo crónico de cannabis (Duperrouzel, 2020; Figueiredo et al., 2020). Mientras que algunos estudios informan un deterioro neuropsicológico a largo plazo derivado de los efectos neurotóxicos del cannabis (Meier et al., 2012), otras investigaciones sugieren que el consumo de cannabis en la adolescencia no parece disminuir el cociente de inteligencia o la función ejecutiva excepto la memoria de trabajo espacial (Meier et al., 2018). Sin embargo, varios estudios muestran una amplia variedad de grados de disfunción cognitiva en pacientes dependientes del alcohol que van desde el DCL hasta la demencia, especialmente de inicio temprano (Sachdeva et al., 2016; Schwarzinger et al., 2018b). Más tarde comentaremos los niveles plasmáticos de NfLs y BDNF según el tipo de droga que respaldan estas investigaciones.

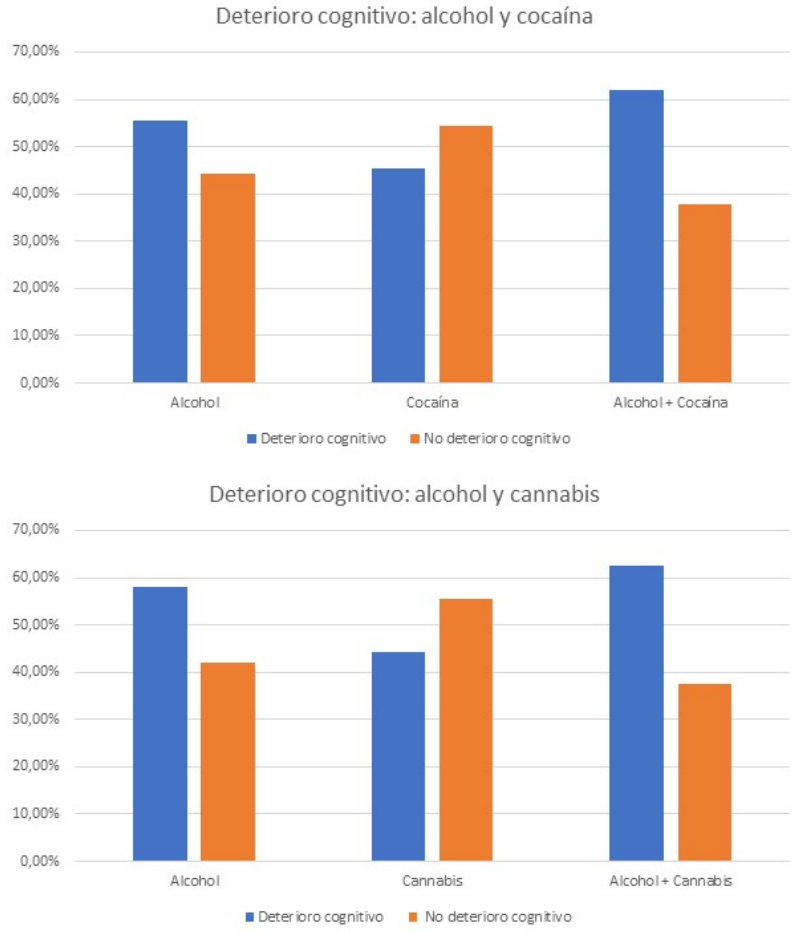

Figura 22. Déficit en función frontal según el tipo de TUS (alcohol, cocaína, cannabis). Resultados analizados a partir de los datos recopilados del Estudio 4.

5.3. Reserva cognitiva y trastornos por uso de sustancias

La reserva cognitiva es entendida como un mecanismo activo para enfrentar el daño cerebral que depende de la creación de redes neuronales flexibles y efectivas como resultado de la estimulación cognitiva a lo largo de la vida (Stern, 2013). En los estudios clínicos de la presente tesis elegimos el nivel educativo alcanzado de los pacientes para evaluar el nivel de RC ya que es una de las medidas más utilizadas al permitir inferir el resto de factores (ej. inteligencia innata, ocupación, nivel socioeconómico, etc.) (Clare et al., 2017). De este modo, la integridad cognitiva global se ha relacionado mayoritariamente con los años de educación y en menor medida con las actividades de ocio, mientras que las actividades laborales se han asociado con la planificación y la respuesta inhibitoria visuoespacial en pacientes policonsumidores (A. Toledo-Fernández et al., 2020).

Reserva cognitiva y variables relacionadas con la adicción:

Una revisión reciente ha descrito el papel preventivo de la RC sobre el inicio del consumo de drogas, el desarrollo de la adicción, la gravedad de los problemas relacionados con las sustancias y mejores resultados de los tratamientos (Cutuli et al., 2019). Los resultados de los estudios de la presente tesis sitúan el nivel educativo como un factor protector o de riesgo en función de los logros académicos, ocupando un papel fundamental en la aparición y evolución de la adicción al alcohol y la cocaína. De este modo, el inicio del consumo y el desarrollo del trastorno por uso alcohol y cocaína se producen de manera precoz en los pacientes que tienen un nivel educativo bajo, mientras que se observa el efecto contrario en aquellos que muestran un nivel educativo elevado. Además, los pacientes con trastorno por uso de cocaína con un bajo nivel educativo padecen durante más años el trastorno que aquellos que tienen un nivel educativo elevado (Tabla 8). En consonancia con nuestros resultados, los jóvenes que abandonan la escuela secundaria o la universidad tienen mayor riesgo de desarrollar abuso de alcohol en la vida adulta en comparación con aquellos que completan estos estudios (Crum et al., 1998). Asimismo, la

literatura científica sugiere que el nivel educativo es un factor determinante en la progresión del abuso y la dependencia de sustancias ilícitas en adolescentes (Strong et al., 2016).

Tabla 8. Influencia del nivel educativo sobre las variables relacionadas con la adicción a la cocaína y al alcohol. Las Tablas están adaptadas de los Estudios 1 y 2.

Variables	Trastorno por consumo de alcohol N =58					
	Elemental N = 22	Secundario N = 25	Universidad N = 11	ANCOVA (estadísticas) [a]		
				valor F	d.f.	valor *p*
Edad de inicio del consumo [media (DE)] (años)	14,15 (2,28)	15.24 (2.09)	17,36 (4,48)	3.175	2.56	**0.050**
Edad de desarrollo de la dependencia [media (DE)] (años)	26,25 (8,51)	32.24 (12.03)	38 (9.32)	4.994	2.52	**0.010**
Duración del diagnóstico de AUD [media (DE)] (años)	16,15 (10,93)	19.56 (18.86)	9.45 (8.25)	0.176	2.53	0.893
Criterios de gravedad [media (rango)]						
Criterios	8 (2.07)	8 (2.06)	7.36 [2–11]	0.364	2.57	0.697
Duración de la abstinencia [media (rango)] (días)	140,95 [0–1270]	149,92 [14–149,92]	56,64 [14–120]	1.424	2.56	0.250

Variable		Trastorno por consumo de cocaína N = 272			
		Primaria N = 69	Secundario N = 178	Universidad N = 25	Valor *p*
Criterios de gravedad [media (95% IC)]	Criterios (0–11)	8,03 (7,32–8,74)	8,17 (7,70–8,63)	7,85 (6,73–8,97)	0.473
Edad de inicio de consumo [media (DE)]	Años	18,33 (5,76)	20.35 (7.04)	24,93 (8,28)	**0.016**
Edad de desarrollo de la dependencia [media (DE)]	Años	24,12 (6,88)	26.01 (7.20)	30,64 (7,88)	**0.001**
Duración de la abstinencia [media (DE)]	Días	199,42 (454,97)	173,56 (681,67)	219,75 (316,75)	0.127
Número de abstinencias [media (DE)]	Número	1,40 (0,89)	1,44 (0,933)	2,00 (1,25)	0.074
Duración de CUD [media (DE)]	Años	10.28 (9.04)	6.81 (5.34)	6.52 (5.52)	**0.020**
Efecto telescópico [media (DE)]	Años	5.06 (6.16)	5.88 (5.18)	6.71 (7.52)	0.157

Reserva cognitiva y prevención del deterioro cognitivo:

La RC también se ha relacionado con un mejor rendimiento cognitivo y menores indicadores de trastorno neurocognitivo (Aldebarán Toledo-Fernández et al., 2019). De este modo, se ha demostrado que un alto nivel educativo suaviza las manifestaciones clínicas y la progresión del DCL (Mazzeo et al., 2019; Soldan et al., 2017). En consonancia, encontramos que los pacientes TUA con un nivel educativo de primaria presentan puntuaciones en función frontal (FAB) más bajas que aquellos que tienen nivel educativo universitario aun controlando la variable edad (Figura 23). Esto nos indica que los pacientes con estudios universitarios están protegidos frente al deterioro cognitivo causado por el consumo de alcohol a lo largo de la vida, mientras que los pacientes con estudios primarios muestran una especial vulnerabilidad a la aparición de déficits frontales. En esta línea, el

consumo de cantidades elevadas de alcohol se ha asociado con un peor rendimiento cognitivo en pacientes de bajo nivel socioeconómico (Sabia et al., 2011). Además, un estudio longitudinal indicó que tener un nivel educativo inferior a la escuela secundaria y una ocupación poco cualificada se asocian con un mayor riesgo de demencia en personas que consumen alcohol (Sabia et al., 2018).

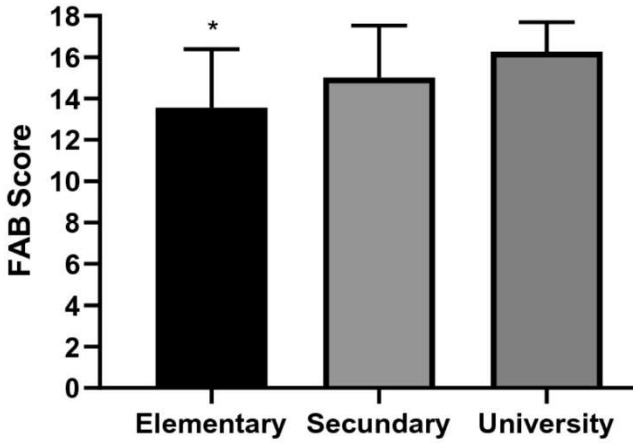

Figura 23. Puntuaciones en función frontal (FAB) según el nivel educativo de los pacientes TUA. Figura extraída del Estudio 1.

Reserva cognitiva y potenciales biomarcadores:

Estudios preclínicos y clínicos han analizado la presencia de biomarcadores para dilucidar qué factores relacionados con la RC amortiguan el deterioro cognitivo derivado del envejecimiento normal o de diversas neuropatologías (Barulli & Stern, 2013). No obstante, y en base al análisis hecho de la literatura publicada, nos encontramos muy lejos de conocer qué biomarcadores podrían asociarse con una RC protectora versus a aquellos que se podrían asociar a un RC que facilitase la progresión patológica hacia deterioro cognitivo. Por eso, este fue uno de nuestros objetivos fundamentales de la presente tesis. Nuestros resultados indican que los pacientes con un bajo nivel educativo tuvieron mayores concentraciones plasmáticas de NT-3. Este resultado podría sugerir un intento fallido del sistema para compensar el daño cerebral mediante la neurogénesis en lugar de implementar mecanismos compensatorios cerebrales más efectivos [por ejemplo, aquellos derivados del aprendizaje y capacitación continuos a lo largo del progreso en el sistema educativo y que proporcionarían una mayor RC (Figura 24)]. Además, las concentraciones plasmáticas de

NT-3, la edad de inicio del consumo y la edad de desarrollo de la dependencia conforman un modelo con una buena capacidad para discriminar entre pacientes con una educación primaria de una educación universitaria en un 91,8%. Es importante señalar que este es el primer estudio científico que posiciona la NT-3 como un potencial biomarcador de RC, por lo que se requiere más investigación al respecto en un futuro.

A pesar de que estudios clínicos y preclínicos posicionan el BDNF como un biomarcador potencial para describir las bases neurobiológicas de la RC en animales y humanos (Fares et al., 2013; Griñan-Ferré et al., 2016), no encontramos alteraciones en las concentraciones plasmáticas de BDNF en función del nivel educativo. No obstante, podría actuar junto con la NT-3 como señal compensatoria contra el deterioro cognitivo asociado al alcohol. En consonancia, existe una línea emergente que apoya el papel de la RC y la vía de señalización BDNF/TrkB como respuesta compensatoria retrasando los síntomas en estadios tempranos de la enfermedad de Alzheimer (Kao et al., 2012; Mazzeo et al., 2019; Patterson, 2015; Soldan et al., 2017).

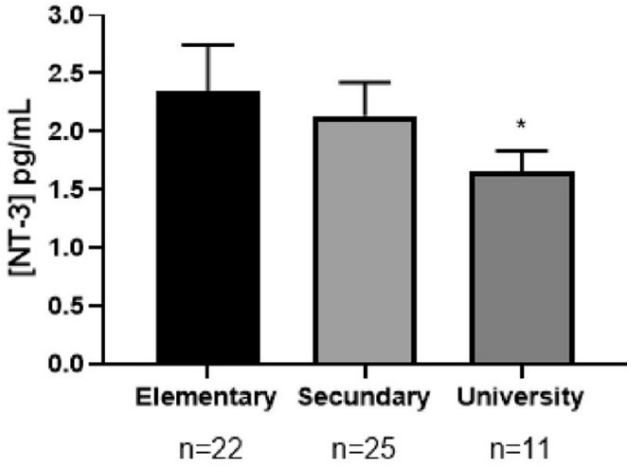

Figura 24. Niveles plasmáticos de NT-3 según el nivel educativo de los pacientes TUA. Figura extraída del Estudio 1.

5.4. Factores de crecimiento, deterioro cognitivo y alcohol

Factor neurotrófico derivado del cerebro (BDNF):

Existe una extensa literatura científica sobre el comportamiento de BDNF bajo condiciones de consumo excesivo de alcohol. En general, las investigaciones preclínicas sugieren que la vía del BDNF corticostriatal juega un papel crucial en la regulación del consumo de alcohol, manteniéndolo moderado a través de la activación de la vía de señalización del BDNF dentro del cuerpo estriado dorsolateral (Logrip ML, Janak PH, 2009; Orrù A, Caffino L, Moro F, Cassina C, Giannotti G, Di Clemente A & F, 2016). No obstante, cuando se reducen los niveles de BDNF en el córtex prefrontal medial se inicia la transición de un consumo moderado a elevado (Logrip et al., 2015). Asimismo, se conoce que la exposición a largo plazo del alcohol en modelos animales da como resultado la pérdida de células corticales y del hipocampo, así como la reducción BDNF en esta última región, lo cual es esencial para la supervivencia neuronal y la función cognitiva (Vetreno, R. P., Hall, J. M., & Savage, 2011). Sin embargo, se requieren más estudios clínicos acerca de cuál es el papel de esta neurotrofina en el deterioro cognitivo en pacientes con consumo crónico de alcohol a lo largo de la vida.

De este modo, los estudios de la presente tesis demuestran que los niveles plasmáticos de BDNF se encuentran reducidos en pacientes con diagnóstico de TUS en comparación con un grupo control y un grupo de demencias. Además, se observó menores concentraciones de BDNF en aquellos que presentaron un deterioro cognitivo severo en comparación con aquellos que no tenían deterioro cognitivo. Más aún, los bajos niveles de BDNF en el grupo TUS se debieron a aquellos pacientes con un diagnóstico de TUA por sí solo o junto con otro TUS como el trastorno por uso de cocaína y cannabis (Figura 25). Además, obtuvimos menores concentraciones de BDNF en los pacientes TUA con deterioro cognitivo frente a los pacientes TUA no deteriorados cognitivamente. Por otro lado, pudimos demostrar que las concentraciones plasmáticas de BDNF, la edad de inicio del consumo, la duración del diagnóstico de TUA, los criterios de gravedad y la duración de la abstinencia pueden clasificar en un 88,2% a pacientes TUA con y sin deterioro cognitivo.

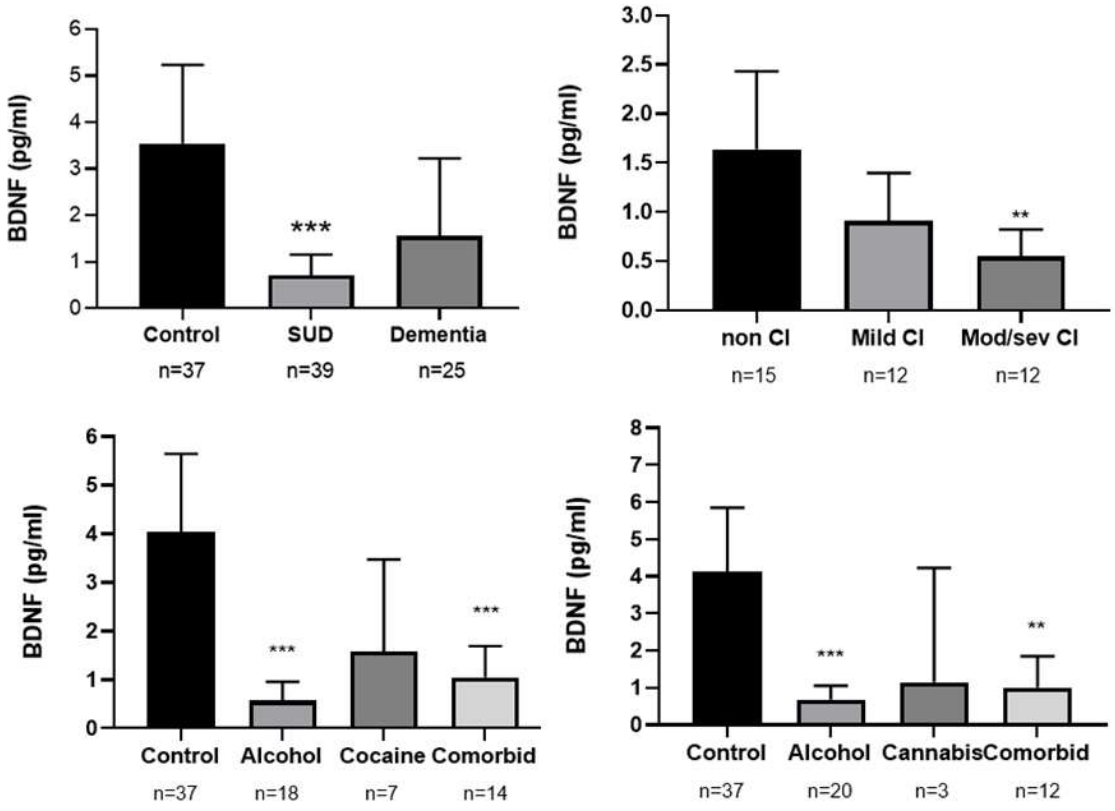

Figura 25. Niveles plasmáticos de BDNF y deterioro cognitivo en pacientes con TUS y TUA. Figura extraída del Estudio 4. Abreviaturas en inglés: SUD = Trastorno por Uso de Sustancias, CI = Deterioro cognitivo.

Por último, los resultados de la presente tesis posicionan el BDNF como una señal íntimamente ligada con el daño neuroaxonal. Las concentraciones de BDNF se correlacionan negativamente con las concentraciones plasmáticas de NfLs en los pacientes con TUS y en el grupo control, pero no en el grupo de demencia. Lo cual quiere decir que cuando se produce el inicio de la neurodegeneración al mismo tiempo se acompaña de una bajada de factores neuroprotectores. Además, observamos que la relación NfL/BDNF era mayor en el grupo TUS y el grupo de demencia que en el grupo control, lo que podría significar una clara señal de deterioro cognitivo derivado de un biomarcador de apoptosis neuronal junto con un biomarcador de neurogénesis (Figura 25). En otras palabras, el consumo de alcohol de por vida no solo es neurotóxico, sino también antirregenerativo al dañar gravemente las señales tróficas mediadas por moléculas como el BDNF. Sería muy

importante profundizar que otros factores tróficos (FCN, GDNF, CNTF, etc.) podrían verse dañados por el consumo crónico de alcohol.

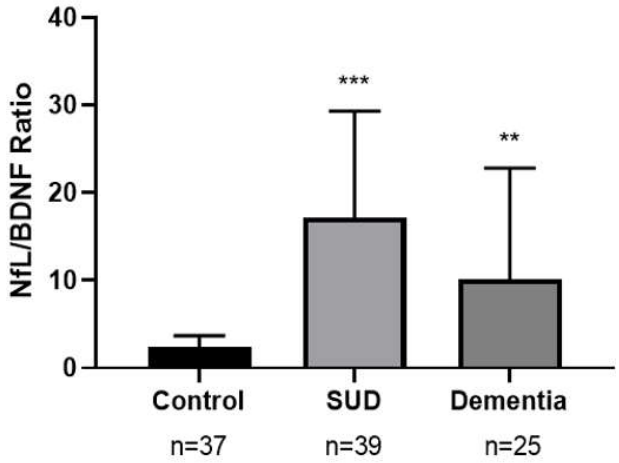

Figura 25. BDNF como señal ligada a al daño neuroaxonal. Figura extraída del Estudio 4. Abreviaturas en inglés: SUD = Trastorno por Uso de Sustancias.

Es importante mencionar que estas diferencias encontradas en los niveles plasmáticos de BDNF no están influidas por un problema médico ($F_{1,35}$=1,935; p=0,173) ni psiquiátrico comórbido ($F_{1,35}$=0,272; p=0,605) incluso controlando las variables sexo, edad e IMC (Figura 26). El hecho de que no se encuentren asociaciones significativas entre BDNF, demencia, problemas médicos asociados y comorbilidad psiquiátrica en estos estudios supone un aliciente para nuestra investigación, añadiendo especificidad a esta molécula como biomarcador específico de deterioro cognitivo en adicciones, sobre todo, en el alcoholismo y; por tanto, eliminando la influencia de otras patologías neuropsiquiátricas o enfermedades médicas.

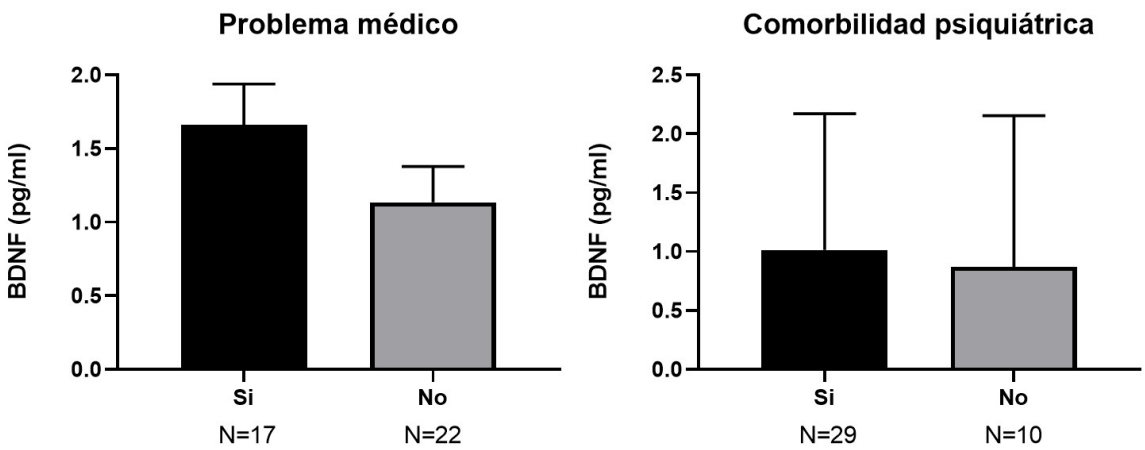

Figura 26. Concentraciones plasmáticas de BDNF en función de los problemas médicos asociados y la comorbilidad psiquiátrica. Resultados analizados a partir de los datos recopilados del Estudio 4.

Factor de crecimiento endotelial vascular:

Debido a que VEGFA juega un papel fundamental en la preservación del estado homeostático neuronal mediante sus funciones angiogénicas y reguladoras de la barrera hematoencefálica, en la presente tesis se quiso explorar este biomarcador en el deterioro cognitivo de los pacientes TUA. De este modo, los resultados arrojaron una correlación negativa significativa entre VEGFA y el desempeño en función frontal (evaluado mediante FAB), así como mayores niveles de VEGFA en el grupo TUA con deterioro cognitivo severo en comparación con el grupo TUA sin deterioro cognitivo (Figura 27). Esto podría indicar que el detrimento de la función cognitiva en estos pacientes se relaciona con la presencia de hipoperfusión e hipoxia, al igual que ocurre en otros tipos de demencia (Chiappelli, 2006; Tarkowski, 2002). No obstante, es importante mencionar que encontramos alteraciones en VEGFA ante la presencia de trastornos psiquiátricos comórbidos con el TUA.

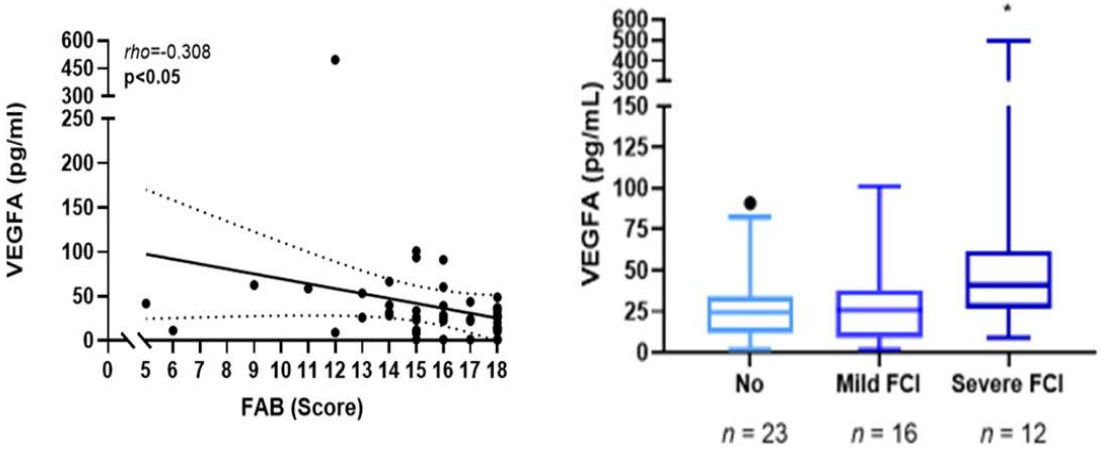

Figura 27. VEGFA y función cognitiva frontal en pacientes TUA. Abreviaturas en inglés: FCI = Deterioro cognitivo frontal. Figuras extraídas del Estudio 3.

Además, observamos que los aumentos de VEGFA se asocian con la elevación de quimioquinas en los pacientes con deterioro cognitivo, pero no en los pacientes sin

deterioro cognitivo. Esto podría sugerir un aumento en la permeabilidad de la barrera hematoencefálica que permite la penetración cerebral de citoquinas, quimioquinas y la infiltración de células inmunes (Louboutin et al., 2012; Muneer et al., 2012), produciendo un estado neuroinflamatorio y la alteración de la cognición (Argaw et al., 2012) (Tabla 9).

Tabla 9. Correlaciones entre VEGFA y quimioquinas en pacientes TUA con y sin deterioro cognitivo.

VARIABLES	VEGFA	
	Cognitive impairment N=30	No cognitive impairment N=20
SDF-1 *[Rho]*	**0.815****	**0.452***
Eotaxin *[Rho]*	**0.715****	0.337
MIP-1α *[Rho]*	**0.508****	-0.179
MCP-1 *[Rho]*	**0.651****	0.207
Fractalkine *[Rho]*	**0.729****	-
VEGFA *[Rho]*	-0.005	0.357

5.5. Quimioquinas, deterioro cognitivo y alcohol.

Asimismo, quisimos explorar si la etiología del deterioro cognitivo en los pacientes TUA podría derivarse de la neuroinflamación que produce el alcohol sobre el sistema inmune a través de la estimulación de los receptores TLR4 de las microglía o a partir de señales inflamatorias que proceden de tejidos periféricos y que acaban ingresando en el cerebro (Banks, 2015; González-Reimers et al., 2014; Montesinos et al., 2016). Sin embargo, los resultados de la presente tesis únicamente arrojan una correlación negativa entre las concentraciones plasmáticas de fractalquina y el desempeño en función frontal en los pacientes TUA. En la misma línea, algunos estudios han informado que los pacientes con enfermedad de Alzheimer de leve a moderada tenían niveles plasmáticos más altos de fractalquina que aquellos con enfermedad de Alzheimer grave (Perea et al., 2018; Strobel et

al., 2015). No obstante, es importante señalar que los pacientes TUA con deterioro cognitivo podrían experimentar una situación neuroinflamatoria debido a los aumentos en VEGFA como se ha mencionado anteriormente.

5.6. Neurofilamentos, demencia de inicio temprano y alcohol

Para demostrar los efectos directos del alcohol sobre el daño neuronal y el inicio de demencia temprana medimos evaluamos las concentraciones de NfLs en plasma, un biomarcador robusto para detectar enfermedades neurodegenerativas que está estrechamente relacionado con la senescencia (Khalil et al., 2018).

Muchos informes que abordan el riesgo de demencia, como la Comisión Lancet sobre prevención, intervención y atención de la demencia, están comenzando a destacar el TUA como un factor de riesgo atribuible sustancial para la demencia en estos últimos años (Livingston et al., 2020). El enfoque sobre los efectos protectores potenciales del consumo moderado de alcohol probablemente ha complicado el análisis y la interpretación de los hallazgos previos, y probablemente se ha pasado por alto la importancia potencial y el efecto del consumo excesivo de alcohol como factor de riesgo modificable para la demencia. De este modo, los artículos que posicionan el alcohol como un potente factor de riesgo desencadenante de todo tipo de demencias e incluso la demencia alcohólica han recibido dos críticas importantes:

1) Estos artículos se han centrado en el diagnóstico de TUA en lugar de diferenciar entre los distintos niveles de consumo de alcohol. Por lo tanto, se necesita aclarar si existe una asociación entre la magnitud de los volúmenes de consumo de alcohol y el riesgo de demencia.

2) La falta de análisis sobre la relación entre el alcohol y los factores/comorbilidades concomitantes (mala alimentación y estilo de vida, tabaquismo, comorbilidad cardiovascular, menor adherencia a los tratamientos médicos, depresión y potencial aislamiento social). Por ello, es necesario aclarar si realmente los problemas

asociados al consumo de alcohol como la comorbilidad médica y psiquiátrica son los que impulsan el riesgo de demencia.

Con respecto al primer punto, los estudios epidemiológicos apuntan a que solo en condiciones de un consumo exacerbado de alcohol prolongado en el tiempo y el desarrollo del TUA acaban desencadenando demencia. De este modo, parece que el consumo excesivo de alcohol en personas sin diagnóstico de TUA no sería suficiente para alterar los niveles de NfLs a pesar de encontrarse evidencias de alteraciones en neurológicas en neuroimagen. Sin embargo, nuestros resultados apoyan que el desarrollo del TUA puede llegar a producir daño neuroaxonal, antiregeneración neuronal y alteraciones en la función cognitiva.

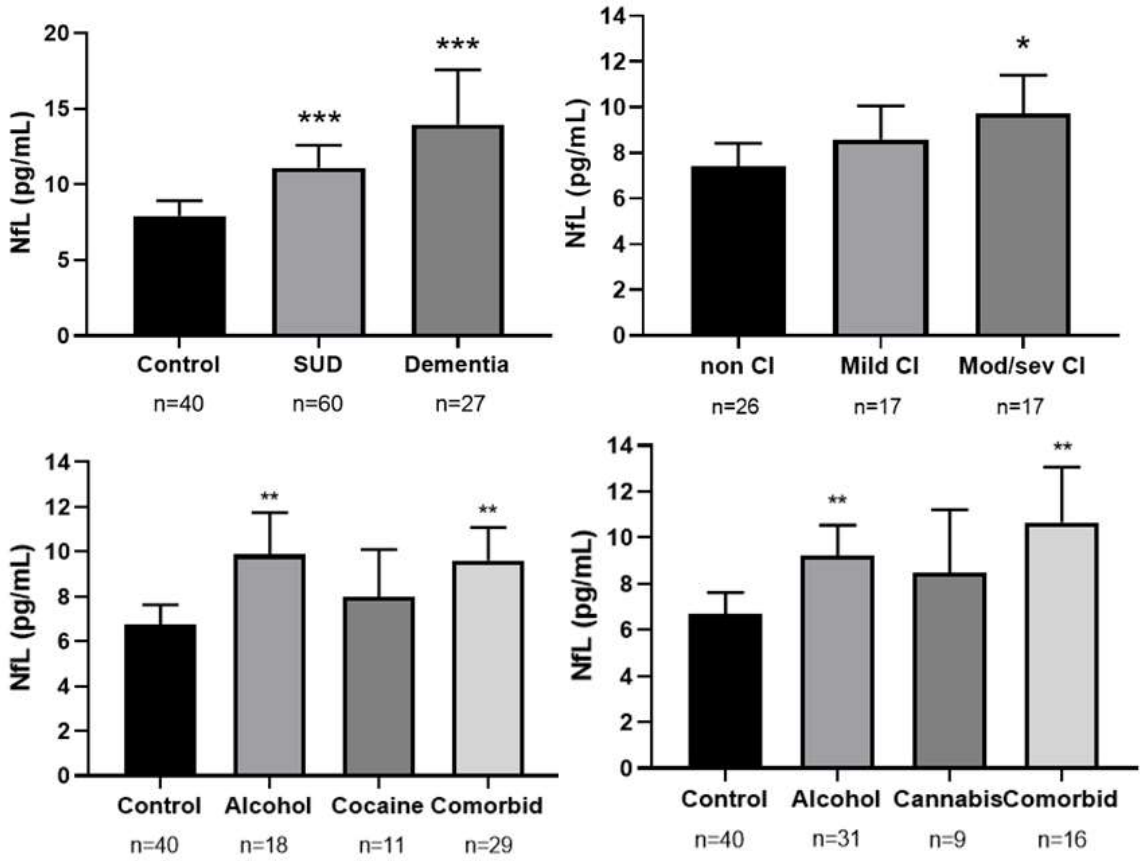

Figura 28. Niveles plasmáticos de NfLs en el TUS. Figuras extraídas del estudio 4. Abreviaturas en inglés: SUD = Trastorno por Uso de Sustancias, CI = Deterioro cognitivo.

De este modo, los resultados de la tesis avalan que las concentraciones NfLs son mayores en los pacientes TUS en comparación con sujetos sanos y, estas diferencias en NfLs se asemejan a las que se encuentran en los pacientes con algún tipo de demencia. Este

efecto sobre los niveles de NfLs se observa cuando los pacientes con TUS presentan un deterioro cognitivo moderado-severo, pero no si es de carácter leve. Además, es importante mencionar que solo se encuentran alteraciones en los niveles de NfLs en los pacientes con un diagnóstico de TUA, pero no en pacientes con trastorno por uso de cocaína o cannabis por sí solo, aunque la comorbilidad de estos dos últimos con el TUA parece potenciar este efecto (Figura 28). Por otro lado, las variables edad, NfLs, BDNF, edad de inicio del TUA, duración del diagnóstico de TUA y gravedad de la adicción al alcohol pueden clasificar a los pacientes deteriorados de los no deteriorados en un 94,6%. Por lo tanto, estos resultados implican una evidencia directa de que el consumo excesivo de alcohol a lo largo del tiempo y el desarrollo del TUA pueden producir una demencia neurodegenerativa de inicio temprano.

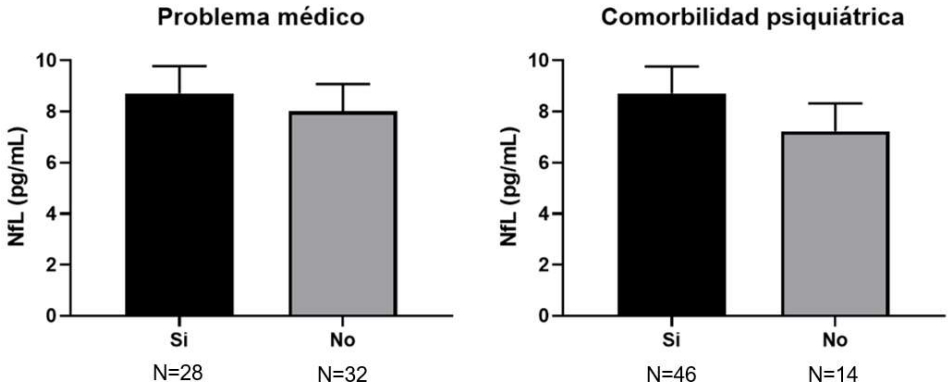

Figura 29. Concentraciones plasmáticas de NfLs en función de los problemas médicos asociados y la comorbilidad psiquiátrica. Resultados analizados a partir de los datos recopilados del Estudio 4.

Con respecto al segundo punto, la detección de NfLs se ha relacionado con las enfermedades neurodegenerativas y no con las enfermedades psiquiátricas (Ashton et al., 2021). En consonancia, los niveles de NfLs en los pacientes TUA no se relacionan con la presencia de problemas médicos ($F_{1,60} = 0,779$, $p = 0,381$) ni comorbilidad psiquiátrica ($F_{1,60} = 3,096$, $p = 0,084$) ni aun cuando se controlan las variables sexo, edad e IMC (Figura 29). Por lo tanto, se descarta la posibilidad de que el riesgo de demencia se deba a otros

factores/comorbilidades concomitantes con el consumo de alcohol, atribuyendo el daño neuroaxonal directamente al TUA.

De este modo, los hallazgos de este estudio son novedosos y brindan la oportunidad de redefinir nuestra comprensión sobre los riesgos que supone el consumo de alcohol, dando un mensaje claro de salud pública y desarrollando estrategias de prevención óptimas para el deterioro cognitivo y la demencia relacionados con el TUA.

6. Conclusiones

El consumo de excesivo y crónico de alcohol a lo largo de la vida produce el detrimento de la cognición sobre todo en las funciones ejecutivas y en la memoria episódica verbal. La fisiopatología subyacente al deterioro cognitivo observado podría consistir en la desregulación de los factores de crecimiento y la señalización inmune. De este modo, el aumento de los niveles plasmáticos de VEGFA podría ocasionar la entrada de citoquinas y quimioquinas en el cerebro a través de la apertura de la barrera hematoencefálica, produciendo neuroinflamación y pudiendo desencadenar la muerte neuronal. A este hecho se le suma la bajada de los niveles plasmáticos de BDNF como consecuencia del consumo de alcohol, lo cual puede conducir al fallo en la protección y regeneración neuronal. De este modo, los pacientes quedarían desprovistos de una de las vías de señalización más importantes para hacer frente al daño cerebral, volviéndose probablemente de carácter irreversible. Además, los pacientes TUA no tienen un nivel de escolarización elevado/ alta RC, por lo tanto, son aún más susceptibles de continuar abusando de sustancias y quedan desprovistos de otro mecanismo compensatorio eficaz para hacer frente al daño cerebral, que se manifestaría en un aumento de la NT-3. Como resultado, en los pacientes TUA se produce un daño neuroaxonal progresivo que puede ser observado a través de las concentraciones plasmáticas de NfLs, sobre todo en aquellos que presentan signos de deterioro cognitivo severo, siendo muy similares a las que se dan en otros tipos de demencias. De este modo, podríamos concluir que el consumo crónico y excesivo de

alcohol a lo largo de la vida es capaz de producir deterioro cognitivo que, en algunos pacientes, evolucionaría a una demencia de inicio temprano, sin que intervengan factores como la influencia de problemas médicos o psiquiátricos asociados.

CONCLUSIONES DEL ESTUDIO

I. There is a high prevalence of cognitive impairment in the AUD population, which compromises executive functions and memory.

II. Educational level can be a protective or risk factor in the onset and evolution of alcohol and cocaine addiction: the onset of consumption and the development of alcohol and cocaine use disorder occur early in patients who have a low educational level, while the opposite effect is observed in those who show a high educational level.

III. The educational level may be a protective or risk factor in the appearance of cognitive impairment in AUD patients, which is manifested through increases in plasma concentrations of NT-3 in those with a low educational level.

IV. Plasma levels of BDNF are reduced in AUD patients, especially those with moderate-severe cognitive impairment, and medical and/or psychiatric problems do not influence this association.

V. BDNF has been shown to be a robust biomarker of cognitive impairment associated with chronic alcohol consumption and it is a signal closely linked to neuroaxonal damage.

VI. Plasma levels of VEGFA are elevated in AUD patients with severe cognitive impairment, who may manifest hypoxia and hypoperfusion.

VII. VEGFA increases are related to the elevation of chemokines in AUD patients with cognitive impairment, and it may play an essential role in the brain penetration of these chemokines through their effects on the blood-brain barrier.

VIII. Plasma levels of NfLs are elevated in AUD patients in a similar way to other types of neurological and neurodegenerative problems and this is not due to medical and/or psychiatric problems.

7. Bibliografía

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8. Anexo

Autorización del comité ético

HOJA DE INFORMACION AL PARTICIPANTE

Título del Proyecto de Investigación

PROTEOMICA DE LA ADICCION A COCAINA: BIOMARCADORES CENTRALES Y PERIFERICOS DE ADICCION.

Objetivos de la Investigación

La adicción a cocaína es un problema de salud muy importante en las sociedades europeas, con una alta penetración en edades tempranas y un fuerte impacto social, sanitario y personal. En la actualidad se carece de tratamientos. El presente proyecto pretende explorar métodos para identificar proteínas presentes en el plasma y células sanguíneas de pacientes con dependencia a la cocaína que puedan servir de biomarcadores de adicción y o de severidad en el consumo. EL fin último del proyecto es la mejora en el diagnóstico y atención a la población adicta a cocaína de nuestro entorno social.
Para ello quisiéramos que participara en este estudio en el cual realizaremos una evaluación completa de su adicción a la cocaína y recabaremos información de otros problemas de salud que pueda tener. La evaluación la realiza un Psicólogo especializado. La evaluación psicopatológica se realizará a través de cuestionarios estructurados e instrumentos diagnósticos e incluirá: variables demográficas y psicológicas de interés; comorbilidad psiquiátrica y antecedentes familiares y personales de salud mental; variables relacionadas con consumo: historia de consumo, edad de inicio, tipo, frecuencia, tiempo de consumo, intentos de cesación; tratamientos previos; poli consumo; uso concomitante de alcohol y factores sociales ambientales asociados al consumo.
Además, se le pedirá que proporcione una muestra de sangre 30 ml. Esta sangre será utilizada para determinar marcadores biológicos de la enfermedad. Una parte se utilizará para análisis de RNA y de proteínas en la sangre. Parte de su sangre se almacenará en el banco de muestras de la Red de Trastornos Adictivos para estudios posteriores.

Beneficios

Esta es una donación altruista en el sentido que el participante no obtendrá ninguna compensación económica ni beneficio directo de esta investigación. Sin embargo, esperamos que los resultados obtenidos en esta investigación ayuden a otras personas en el futuro.

Riesgos

Aunque extraer una muestra de sangre no causa problemas serios para la mayoría de las personas, podría causar algún tipo de sangrado, cardenal, y/o molestia en el lugar de la inyección. Cualquier implicación médica adversa como resultado directo de la extracción de la muestra de sangre será tratada bajo la supervisión de los médicos del estudio.

Su participación en este proyecto es voluntaria. Usted puede terminar con el uso de su muestra en cualquier momento sin necesidad de dar ninguna explicación. Si quiere que su muestra sea destruida, por favor escriba una carta firmada declarando su deseo al investigador principal del estudio: Dr. Fernando Rodríguez de Fonseca, dirección postal: Av. Carlos haya 82, pabellón de gobierno. Sótano. CP:29010, Málaga, España.

Deberá de hacer este requerimiento por escrito para conseguir que su muestra sea destruida. Los experimentos que se estén ya realizando cuando se reciba su requerimiento (o aquellos ya completados), continuaran siendo utilizados como parte del proyecto de investigación. En algunas circunstancias, por ejemplo, si las agencias reguladoras del lugar del estudio no requieren seguir manteniendo los datos del estudio, sus datos podrían ser destruidos. Si sus datos han sido destruidos, ya no habrá ninguna conexión entre su información personal y su muestra de sangre y, por tanto, no podrá requerir la destrucción de su muestra.

Protección de su información personal

Tanto la información personal como médica recogida de usted, así como los resultados obtenidos de la investigación de su muestra serán tratados confidencialmente de acuerdo con las leyes de protección de datos (Ley Orgánica de Protección de Datos de carácter personal, 15/1999).

No se recogerá información adicional a la contenida en los cuestionarios que usted completará y aquella información médica que usted decida dar durante su evaluación.

Si usted accede a proveer sangre, en el momento de la recogida de su sangre, la muestra será marcada con un número de identificación único o número "código". La muestra no será marcada con ninguna información personal que le identifique a usted directamente como por ejemplo su nombre, dirección o número de teléfono. Cualquier referencia a las muestras en el transcurso del proyecto de investigación, incluido su almacenamiento, manejo e interpretación de los datos, se realizará solamente mediante el uso de los números codificados. La información médica que facilite como parte de los cuestionarios y la evaluación será asociada con su código, pero no con ningún tipo de información personal que permita identificarla directamente.

La información médica que facilite y los resultados de la investigación derivados de su muestra podrán ser compartidos libremente entre los investigadores.

Posesión de las muestras

Su muestra, identificada sólo con su número de código, podrá ser almacenada hasta 5 años, momento en el que será físicamente destruida. Parte de la muestra será almacenada en el banco de muestras de la Red de Trastornos Adictivos de la que el Investigador Principal de este proyecto es el coordinador.

Contacto del Biobanco:

Carmen de Felipe, Macarena Herrera, Luis Navarro

Tlf: 96 591 9553 / 96 591 9336

E-mail: biobancorta@umh.es

¿Tenemos su permiso para almacenar su muestra codificada, y su información personal y clínica, y a utilizarla en los proyectos de investigación descritos anteriormente llevados a cabo de acuerdo con la normativa legal sobre ética en investigación médica?

SI / NO

Para poder utilizar su muestra codificada en otras investigaciones será necesario un nuevo consentimiento informado. ¿Podemos contactarle en el futuro para otras investigaciones?

SI / NO

Contacto

Si usted necesita información adicional respecto al progreso del proyecto de investigación, si quiere comunicar cualquier cambio de su dirección postal, o retirarse de este proyecto de investigación, puede usted contactar al investigador principal del estudio:

Dr. Fernando Rodríguez De Fonseca, Teléfono Móvil: 669426548. Fax: 34951440263. Correo electrónico: fernando.rodriguez@fundacionimabis.org.

Consentimiento y autorización

Si usted está de acuerdo en proveer la muestra y su información personal y médica y firma este Consentimiento Informado, usted recibirá una copia fechada para su documentación. No firme este Consentimiento Informado si no se han respondido todas sus preguntas a su satisfacción.

Por favor marque con una "X" y firme:

☐ El doctor del estudio ha explicado la naturaleza y el progreso del proyecto de investigación.

☐ He tenido la oportunidad de preguntar cualquier pregunta sobre el proyecto de investigación y han sido respuestas a mi satisfacción.

☐ He leído y entendido completamente este Consentimiento Informado.

☐ **Acuerdo participar en este proyecto de investigación y consiento proveer una muestra de sangre e información personal y médica bajo las condiciones descritas en este Consentimiento Informado.**

☐ Autorizo el uso y divulgación de mi información médica a las partes descritas en este Consentimiento Informado para los fines en él descritos.

☐ Informaré a las investigadoras del estudio de cualquier cambio de dirección.

Nombre (escrito):

_____

Firma Fecha

Persona que realiza el reclutamiento Dr./Dra. _____

Firma_____

Fecha_____

Participación en publicaciones científicas con metodología similar:

Asociación potencial de especies de ácido lisofosfatídico (LPA) plasmático con deterioro cognitivo en pacientes abstinentes ambulatorios con trastorno por uso de alcohol

“Potential association of plasma lysophosphatidic acid (LPA) species with cognitive impairment in abstinent alcohol use disorder outpatients”

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Potential association of plasma lysophosphatidic acid (LPA) species with cognitive impairment in abstinent alcohol use disorders outpatients

Nuria García-Marchena[1,2,8]✉, Nieves Pizarro[3,8], Francisco J. Pavón[1,4,5], Miriam Martínez-Huélamo[3,6], María Flores-López[1], Nerea Requena-Ocaña[1], Pedro Araos[1,7], Daniel Silva-Peña[1], Juan Suárez[1], Luis J. Santín[7], Rafael de la Torre[3]✉, Fernando Rodríguez de Fonseca[1]✉ & Antonia Serrano[1]✉

Lysophosphatidic acid (LPA) species are bioactive lipids participating in neurodevelopmental processes. The aim was to investigate whether the relevant species of LPA were associated with clinical features of alcohol addiction. A total of 55 abstinent alcohol use disorder (AUD) patients were compared with 34 age/sex/body mass index-matched controls. Concentrations of total LPA and 16:0-LPA, 18:0-LPA, 18:1-LPA, 18:2-LPA and 20:4-LPA species were quantified and correlated with neuroplasticity-associated growth factors including brain derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1) and IGF-2, and neurotrophin-3 (NT-3). AUD patients showed dysexecutive syndrome (22.4%) and memory impairment (32.6%). Total LPA, 16:0-LPA, 18:0-LPA and 18:1-LPA concentrations, were decreased in the AUD group compared to control group. Total LPA, 16:0-LPA, 18:2-LPA and 20:4-LPA concentrations were decreased in men compared to women. Frontal lobe functions correlated with plasma LPA species. Alcohol-cognitive impairments could be related with the deregulation of the LPA species, especially in 16:0-LPA, 18:1-LPA and 20:4-LPA. Concentrations of BDNF correlated with total LPA, 18:2-LPA and 20:4-LPA species. The relation between LPA species and BDNF is interesting in plasticity and neurogenesis functions, their involvement in AUD might serve as a biomarker of cognitive impairment.

Alcohol use disorders (AUD) are one of the main global health problems, with a worldwide impact on individuals and society. They cause a significant medical burden in prevention and treatment effort[1,2]. Among the medical consequences related to alcohol dependence we can highlight the prevalence of nutritional deficiencies, hepatic and liver damage[3,4] and the appearance of comorbid psychopathological lifetime complications[5]. Moreover, cognitive impairment associated to AUD is considered one of the main factors for the development of any type

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of dementia[6]. The presence of comorbid medical conditions during AUD could contribute to the lack of control over drinking aggravating the medical treatment[7].

While major depressive disorders and anxiety are the most prevalent psychopathological comorbid disorders in alcohol use population[8–10], cocaine and cannabis abuse are the most common lifetime comorbid substance disorders associated to AUD[11]. Moreover, chronic alcohol consumption could modulate neurogenesis and produce distortions on the central nervous system causing neurocognitive impairment[12,13]. Thus, functional circuits involved on these medical consequences of alcohol include those related to cognition (such as prefrontal cortex-involving circuits) and emotional processing (such as limbic-amigdalar circuits). The most common cognitive alterations in alcohol dependence patient are those related with executive functions, episodic and visuoconstructive memory and emotion[4,14]. In AUD patients, the measurable neuropsychological damage belonging to executive functions have been found impaired in those tasks related to inhibition, flexibility, deduction of rules, organization and planning[15,16]. Recently, we have linked AUD associated with mild and severe cognitive impairment and to a dysfunctional signaling of both, the insulin growth factor (IGF) and the brain derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors[17–19]. These findings suggest that at least partially, monitoring BDNF signaling might help to identify early stages of cognitive impairment in AUD patients. However, in these studies neither IGF-1 nor BDNF were found to be associated to any additional comorbidity, especially psychiatric entities such as depressive and psychotic disorders that it is known to contribute to cognitive impairment[17].

In an attempt to further explore biomolecules that might mediate the effects of alcohol on psychiatric comorbidity and cognitive impairment, we focused our research on lysophosphatidic acid (LPA), an ubiquitous signaling lipid involved in neurodevelopment[20,21] and emotional behavior, including anxiety[21,22]. LPA has the simplest structure of all glycerophospholipids in serum and is highly expressed in the brain[23]. The term LPA refers to a group of different chemical species where the glycerophospholipid moiety is associated to several different carbon acyl chains (i. e. 16-, 18- and 20-LPA), being the species mono-unsaturated oleic acid (18:1-LPA), and poly-unsaturated linoleic acid (18:2-LPA) and arachidonic acid (20:4-LPA) the more abundant in human serum[24,25]. LPA participates in brain cortex formation, cell proliferation, differentiation and survival functions through the activation of distinct G-protein-coupled receptors[20,26,27], although there are evidences that LPA participates in apoptosis processes through oxidative stress[28]. In addition, LPA also is involved in adult neurogenesis[29,30]. These important roles in neurodevelopment and plasticity have suggested that abnormalities in LPA signaling might be related to neurodevelopmental disturbances and neuropsychiatric diseases as schizophrenia, Alzheimer's diseases or autism[31,32]. Recent preclinical studies have also linked LPA to depression, since genetically modified animals lacking LPA1 receptors exhibits an anxious-depressive phenotype[33] associated with high alcohol intake[34]. Because of the previously described involvement of LPA in both alcohol intake and depression, and the participation of these lipids in neural plasticity events, it is feasible to suspect a potential role for LPA species in comorbidities associated with AUD.

Since preclinical studies have suggested a potential role of LPA and its LPA1 receptor as vulnerability factors for excessive alcohol intake, depression and cognitive impairment, we further explore whether this association might also be present in AUD patients. Thus, the main aim of this descriptive clinical study was to characterize the plasma concentrations of LPA species in a chronic alcohol dependence context and to explore if they correlate with the associated psychiatric (i.e. affective disorders) and neuropsychological (cognitive impairment) comorbidities. Additionally, because previous studies have reported the existence of sexual dimorphism in the biological activity of LPA with a greater presence in healthy women compared with men[30,35], the present study was performed in male and female patients to examine the plasma concentration of LPA species according to sex.

Materials and methods

Participants and recruitment. The present study included 89 Caucasian volunteers divided into two groups: 55 abstinent alcohol use disorders patients (AUD group) in outpatient treatments and 34 control subjects (Control group) matched by age, body mass index (BMI) and proportion of sex with the alcohol group. Patients were recruited at the *Hospital Universitario 12 de Octubre* (Madrid, Spain) and *Centro provincial de Drogodependencias* (Malaga, Spain). Control participants were included from databases of healthy subjects willing to participate in medical research projects from *Hospital Universitario 12 de Octubre* (Madrid, Spain) and *Hospital Regional Universitario de Malaga* (Málaga, Spain).

To be eligible for the present study, participants had to meet the following inclusion criteria: ≥ 18–65 years of age, and abstinence from alcohol for at least 4 weeks. The exclusion criteria included: personal history of long-term inflammatory diseases or cancer, cognitive or language limitations, pregnant or breast-feeding women, and infectious diseases. With regard the control group, participants with psychiatric disorders in Axis I were also excluded.

Clinical and neuropsychological assessments. Substance use disorders and other psychiatric disorders were diagnosed according to the DSM-IV-TR criteria[36] using the Spanish version of the Psychiatric Research Interview for Substance and Mental Disorders (PRISM)[36,37]. PRISM is a semi-structured interview with good psychometric properties in the evaluation of substance use disorders and in the main psychiatric comorbid disorders related to substance use population[37,38].

The cognitive assessment was performed with two scales, the Spanish Versions of the Frontal Assessment Battery (FAB)[39] and the Memory Failures Everyday (MFE)[40]. The FAB test is useful for a screening of a frontal lobe dysfunction evaluation. The total score was obtained from 0 to 18 evaluating the respectively subdomains: prehension, go-no-go, conflicting, Luria motor and lexical fluency; a cut-off point less than 16 separate normal from mild dysexecutive deficits, and a cut-off point less than 13 separate mild and severe dysexecutive

syndrome[39]. The MFE questionnaire is formed by 30 items and is useful to evaluate the lack of memory in daily life. The cognitive complaints scores over 36 are related with memory deficits[40].

Collection of plasma samples. Plasma sample collection was based on previous studies about lipid mediators in addiction and comorbid disorders[41]. Blood samples were obtained in the morning after fasting for 8–12 h (prior to the psychiatric interviews) by experienced nurses. Venous blood was extracted into 10 mL K_2 EDTA tubes (BD, Franklin Lakes, NJ, USA) and immediately centrifuged at 2200×*g* for 15 min (4 °C) to obtain plasma. The plasma samples were individually assayed to detect infectious diseases using commercial rapid tests for HIV, hepatitis B, and hepatitis C (Strasbourg, Cedex, France). Plasma samples were individually stored at −80 °C until further analyses.

Analysis of LPA species. The LPA species of saturated fatty acids palmitic acid (16:0-LPA) and stearic acid (18:0-LPA), the LPA of the monounsaturated fatty acid oleic acid (18:1-LPA), and the LPA species of the polyunsaturated fatty acids linoleic acid (18:2-LPA) and arachidonic acid (20:4-LPA) were determined using an extraction protocol followed by LC–MS–MS separation and quantification. Briefly, 0.2 mL of plasma were spiked with 100 ng of a mathanolic solution of 17:0-LPA (IS). A liquid–liquid extraction was performed after the addition of 200 µL of butanol. The organic phase was evaporated and reconstituted in 100 µL of mobile phase (80A:20B, see below) prior to analysis.

Stock solutions (100 µg/mL) for each analyte were independently prepared by diluting adequate amounts of standards in methanol. The working solutions were prepared by mixture of the stock solutions and dilution in methanol. The linearity of calibration curves containing the following concentrations for all the target analytes: 0.2, 0.5, 1, 1.5, 2, 4, 6, 8, 10 µg/mL was verified being the coefficient of determination $r^2 > 0.99$ in all cases.

Before the quantification of real samples and in order to verify matrix effect and recovery of the analytical method for each analyte, calibration curves were prepared in both plasma and water samples. In all cases, matrix effects lower than 6% and recoveries higher than 66% were achieved. At this point, calibration curves to perform quantification of real samples were prepared in water, and were added in duplicate in each analytical batch.

The procedure of lipid analysis in plasma was performed by a validated method previously described in clinical samples[41]. Quantification of LPA species in human plasma was performed using an ACQUITY UPLC system (Waters Associates, Milford, MA, USA) for the chromatographic separation coupled to a triple quadrupole (Xevo TQ-S micro) mass spectrometer provided with an orthogonal Z-spray-electrospray interface (ESI) (Waters Associates, Milford, MA, USA). The drying and nebulizing gas was nitrogen. The desolvation gas flow was set to 1200 L/h and the cone gas flow to 50 L/h. A capillary voltage of 3 kV was used in negative ionization mode. The nitrogen desolvation temperature was set to 600 °C and the source temperature to 150 °C. Collision gas was argon and the injection volume was 5 µL.

The chromatographic separation was achieved at 30 °C using an ACQUITY UPLC BEH C18 column (2.1 × 100 mm × 1.7 µm) (Waters Associates, Milford, MA, USA), at a flow rate of 300 µL/min. Mobile phase A was ammonium formate 1 mM with formic acid (0.01% v/v) dissolved in methanol. Mobile phase B was ammonium formate 1 mM with formic acid (0.01% v/v) in water. A gradient program was employed for the separation of the analytes; the percentage of mobile phase B linearly changed as follows: 0 min, 20%; 0.2 min, 20%; 6 min, 10%; 6.5 min, 10%; 7 min, 20%; 8 min, 20%. Total run time was 8 min. Analytes were determined by a Selected Reaction Monitoring (SRM) method by acquiring two transitions for each compound as specified (Supplementary Table S1). The most specific transition was selected for quantitative purposes. MassLynx software V4.1 and TargetLynx XS were used for data management. Finally, the LPA species plasma concentrations were recalculated to molar concentration (nmol/L).

Analysis of neurotrophic factors. Plasma concentrations of BDNF, IGF-2 and NT-3 were determined using different enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions: human BDNF SimpleStep ELISA Kit (#ab212166, Abcam, Cambridge, UK) human IGF-2 Quantikine ELISA Kit (#DG200, R&D Systems, Minneapolis, MN, USA) and NT-3 ELISA Kit (#EHNTF3, ThermoFisher Scientific, Alcobendas, Madrid, Spain). To perform the ELISA protocols we used 50 µL of plasma as described previously[17] and plasma concentrations of IGF-1 were estimated by double antibody radioimmunoassay. Plasma fractions were incubated with 125I-IGF-1 at 4 °C for 24 h with IGF-1 antiserum (UB2-495). In each assay a calibration curve and internal controls were included. The BDNF, IGF-1, IGF-2 and NT-3 plasma concentration were recalculated to molar concentration (nmol/L).

Statistical analysis. All data in the tables are expressed as number and percentage of subjects [N (%)] or mean and standard deviation (SD). The significance of differences in categorical and normal continuous variables was determined using Fisher's exact test (chi-square test) and Student's *t* test, respectively.

Multiple analysis of covariance (ANCOVA) was performed to indicate the relative effects of explanatory variable (i.e., lifetime alcohol use disorders, cognitive impairment) on the plasma concentrations of molecular LPA species, controlling for additional independent variables and covariates (e.g., sex, age and BMI). Because we used factors with two levels and there were not significant interactions between factors in the ANCOVA models, post hoc tests for multiple comparisons were not performed. Correlation analyses were performed using the Spearman's coefficient (rho) (Plasma concentrations of LPA species and MFE or FAB scores) and correlation analyses using the Pearson's coefficient (r) in logarithm (10)-transformation concentrations of LPA species and growth factors (BDNF, IGF-1 and IGF-2, and NT-3) to ensure statistical assumption for positive skewed distribution. The statistical analyses were carried out with the GraphPad Prism version 5.04 (GraphPad

Variables	Total sample N = 89		p value
	Control group N = 34	AUD group N = 55	
Age (mean ± SD)			
Years	46.9 ± 8.9	47.7 ± 7.7	0.643[a]
Body mass index (mean ± SD)			
Kg/m^2	26.8 ± 4.2	26.1 ± 3.8	0.433[a]
Sex [N (%)]			
Women	9 (26.5)	10 (18.2)	0.428[b]
Men	25 (73.5)	45 (81.8)	
Marital status [*N* (%)]			
Single	9 (26.5)	13 (23.6)	**0.040**[b]
Cohabiting	19 (55.9)	17 (30.9)	
Separated	6 (17.6)	23 (41.8)	
Widow	–	2 (3.6)	
Education degree [N (%)]			
Elementary	2 (5.9)	17 (30.9)	**0.014**[b]
Secondary	18 (28.1)	25 (45.5)	
University	14 (41.2)	13 (23.6)	
Occupation [N (%)]			
Employed	34 (100)	42 (41.2)	**< 0.001**[b]
Unemployed	–	60 (58.8)	

Table 1. Socio-demographic characteristics of the sample. *AUD* alcohol use disorders. [a]*p* value was calculated with Student's t test. [b]*p* value was calculated with Fischer's exact test or chi-squared test. Bold values are statistically significant for $p < 0.05$.

Software, San Diego, CA, USA), and IBM SPSS Statistical version 22 (IBM, Armonk, NY, USA). A p value < 0.05 was considered statistically significant.

Ethics statements. Written informed consents were obtained from each participant after a complete description of the study. All the participants had the opportunity to discuss any questions or issues. The study and protocols for recruitment were approved by the Ethics Committee of the Hospital Regional Universitario de Malaga (CP14/00173, CP14/00212 and PI13/02261) in accordance with the Ethical Principles for Medical Research Involving Human Subjects adopted in the Declaration of Helsinki by the World Medical Association (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and Recommendation No. R (97) 5 of the Committee of Ministers to Member States on the Protection of Medical Data (1997), and Spanish data protection act [Regulation (EU) 2016/679 of the European Parliament and of the Council 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation). All collected data were given code numbers in order to maintain privacy and confidentiality.

Results

Table 1 shows a socio-demographic description of the 89 participants of both gender included in this study. We selected 55 patients in abstinence from AUD outpatient programs and 34 healthy control subjects matched for sex, age and BMI with AUD patients. Significant differences were observed between the two sample groups with respect to marital status ($p < 0.05$) education degree ($p < 0.05$) and occupation ($p < 0.001$). The mean age of the AUD group was 48 years and the 82% of the participants were men with a BMI of 26. AUD group displayed higher prevalence of separations and divorces, secondary educational level, and unemployment rate than the control group.

Alcohol-related variables and cognitive deficits in AUD group. The variables related to AUD group were evaluated and described in Table 2. The mean age at first drink of alcohol was 15.3 years, while the average age of the AUD onset was 30.2 years with 14.8 years of problematic alcohol use. The mean of addiction criteria was 8 (based on DSM-5) and they had a length of 79 days of abstinence at the moment of the evaluation.

Regarding other abused substances, a 74.2% of the AUD group were smokers and there was a high prevalence of other substance use disorders (SUDs) (41.8%), being cocaine the most prevalent substance use (22.5%). In addition to the SUDs, there was observed an elevated prevalence of other psychiatric disorders (63.6%). Thus, lifetime mood and anxiety disorders were diagnosed in a 27% and 21.3% of AUD group, respectively. Unlike the control participants, the 85.5% of the abstinent alcohol patients received psychiatric medication during the

Variables	AUD group N = 55
Age at first alcohol use [mean (SD)]	
Years	15.3 (3.1)
Age at onset of AUD [mean (SD)]	
Years	30.2 (10.5)
Length of AUD diagnosis [mean (SD)]	
Years	14.8 (10.0)
AUD severity criteria [mean (range)]	8.3 [1–11]
Length of abstinence [mean (range)]	
Days	79 [30–300]
Smoking [*N* (%)]	42 (74.2)
Comorbid substance use disorders [N (%)]	
Cocaine	20 (22.5)
Cannabis	4 (4.5)
Sedatives	2 (2.2)
Comorbid psychiatric disorders N (%)]	
Mood	24 (27.0)
Anxiety	19 (21.3)
Borderline	5 (5.6)
ADHD	1 (1.1)
Psychotic	8 (7.8)
Antisocial	5 (4.9)
Psychiatric medication [N (%)]	
No	8 (14.5)
Antidepressants	29 (52.7)
Anxiolytics	23 (41.8)
Anticraving	16 (29.1)
Disulfiram [*N* (%)]	39 (70.9)
Frontal cognition deficit (FAB) [N (%)]	
No	35 (63.6)
Mild cognitive impairment	14 (15.7)
Severe impairment deficit	6 (6.7)
Memory deficit (MFE) [N (%)]	
No	26 (29.2)
Mild memory deficit	24 (27.0)
Severe memory deficit	5 (5.6)

Table 2. Clinical characteristics of the AUD group. *AUD* alcohol use disorders, *ADHD* attention deficit hyperactivity disorder.

last 12 months: antidepressants (52.7%), anxiolytics (41.8%) and anticraving (29.1%). Finally, the 70.9% of the AUD group were treated with *disulfiram*.

The neuropsychological evaluation revealed that a 22% of the AUD group showed some deficits related to frontal cognition (assessed with FAB); 33% of them suffer memory deficits (assessed with MFE) and 31% showed some impairment of both frontal cognition and memory deficits.

Plasma concentrations of LPA species in abstinent alcohol patients. The impact of the alcohol dependence was studied in the total sample using a two-way ANCOVA with "group" (AUD group and control group) and "sex" as factors, and age and BMI as covariates (Supplementary Table S2).

Plasma concentration of the total LPA was significantly affected by "group" and by "sex" factors, but there was no interaction effect between both factors. Plasma concentration of total LPA was significantly affected by the factor "group" [$F_{(1,82)} = 4.629$; $p = 0.034$]. There was a significant reduction in total LPA plasma concentration in the AUD group compared with the control group [60.847 (95% CI 52.190–69.503) nmol/L and 74.922 (95% CI 65.190–84.512) nmol/L, respectively]. Regarding LPA species, plasma concentration of 16:0-LPA [$F_{(1,82)} = 5.640$; $p = 0.020$], 18:0-LPA [$F_{(1,82)} = 5.166$; $p = 0.026$] and 18:1-LPA [$F_{(1,82)} = 7.114$; $p = 0.009$] were significantly affected by the factor "group". Specifically, 16:0-LPA, 18:0-LPA and 18:1-LPA plasma concentrations were significantly lower in the AUD group than in the control group [16:0-LPA: 8.094 (95% CI 6.979–9.210) nmol/L and 10.096 (95% CI 8.861–11.332) nmol/L; 18:0-LPA: 2.958 (95% CI 2.727–3.199) nmol/L and 3.372 (95% CI 3.105–3.639) nmol/L; and 18:1-LPA: 5.998 (95% CI 25.258–6.738) nmol/L and 7.489 (95% CI 6.670–8.308) nmol/L; respectively]. By

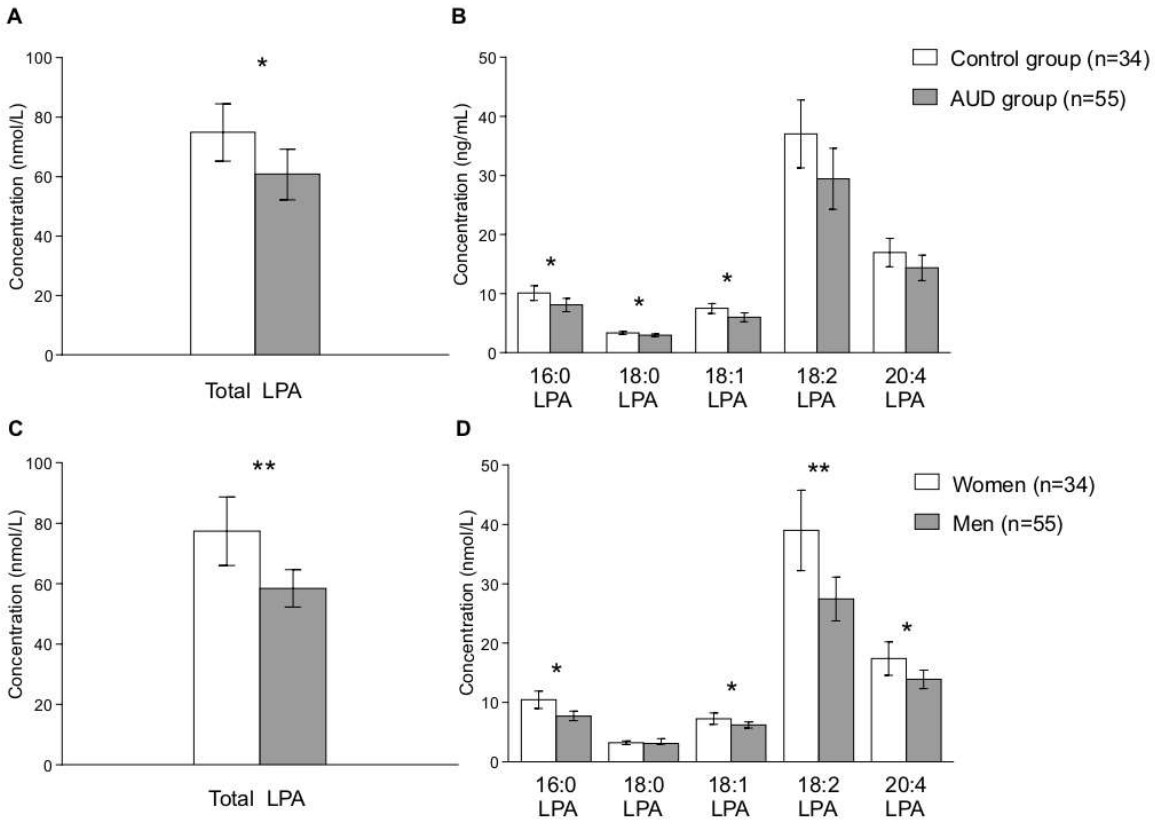

Figure 1. Plasma concentrations of LPA species in the sample according to the group and sex. (**A**) Bars are estimated marginal means and 95% confidence intervals (95%) representing total LPA (nmol/L) according to the group; (**B**) bars are estimated marginal means and 95% confidence intervals (95%) representing LPA species (nmol/L) according to the group; (**C**) bars are estimated marginal means and 95% confidence intervals (95%) representing total LPA (nmol/L) according to the sex; (**D**) bars are estimated marginal means and 95% confidence intervals (95%) representing LPA species (nmol/L) according to the group. Data were analyzed by two-way analysis of covariance (ANCOVA) and *p < 0.05 and **p < 0.010 denote a significant main effect of group factor or sex.

contrast, 18:2-LPA and 20:4-LPA plasma concentrations were not affected by the "group" factor or interaction effect between "group" and "sex" factors.

Regarding the impact of "sex", the plasma concentration of total LPA [$F_{(1,82)} = 8.377$; $p = 0.005$], 16:0-LPA [$F_{(1,82)} = 10.539$; $p = 0.002$], 18:2-LPA [$F_{(1,82)} = 8.755$; $p = 0.004$] and 20:4-LPA [$F_{(1,82)} = 4.548$; $p = 0.036$] were significantly different between men and women. Thus, women had higher total LPA concentrations than men [77.360 (66.023–88.698) nmol/L and 58.409 (95% = 52.205–64.613) nmol/L, respectively]. Similarly, we observed significantly higher concentrations of 16:0-LPA, 18:2-LPA and 20:4-LPA in women than in men [16:0-LPA, 10.465 (9.004–11.926) nmol/L and 7.726 (95% CI 6.926–8.525) nmol/L; 18:2-LPA, 39.012 (95% CI 32.234–45.790) nmol/L and 27.429 (95%CI 23.720–31.138) nmol/L; and 20:4-LPA, 17.405 (95% CI 14.572–20.237) nmol/L and 13.916 (95% CI 12.916–15.466) nmol/L; respectively] (see Supplementary Table S3). Estimated marginal means for "group" and "sex" factors are represented in Fig. 1.

Plasma concentrations of LPA species in comorbid psychiatric disorders in abstinent alcohol patients. As shown in the clinical description of the sample (Table 2), mood and anxiety disorders were the most prevalent comorbid psychiatric disorders in the AUD group. Thus, we examined the effect of mood and anxiety comorbid disorders in total LPA and LPA species in the AUD group using a two-way ANCOVA with "comorbidity/mood or anxiety disorders" (comorbid subgroup and non-comorbid subgroup) and "sex" as factors, and age and BMI as covariates. However, we did not observe main effects or interaction effects on LPA concentrations (Supplementary Table S4, S5).

Plasma concentrations of LPA species in cognitive impairments in abstinent alcohol patients. We investigated cognitive functioning following chronic alcohol consumption, in memory

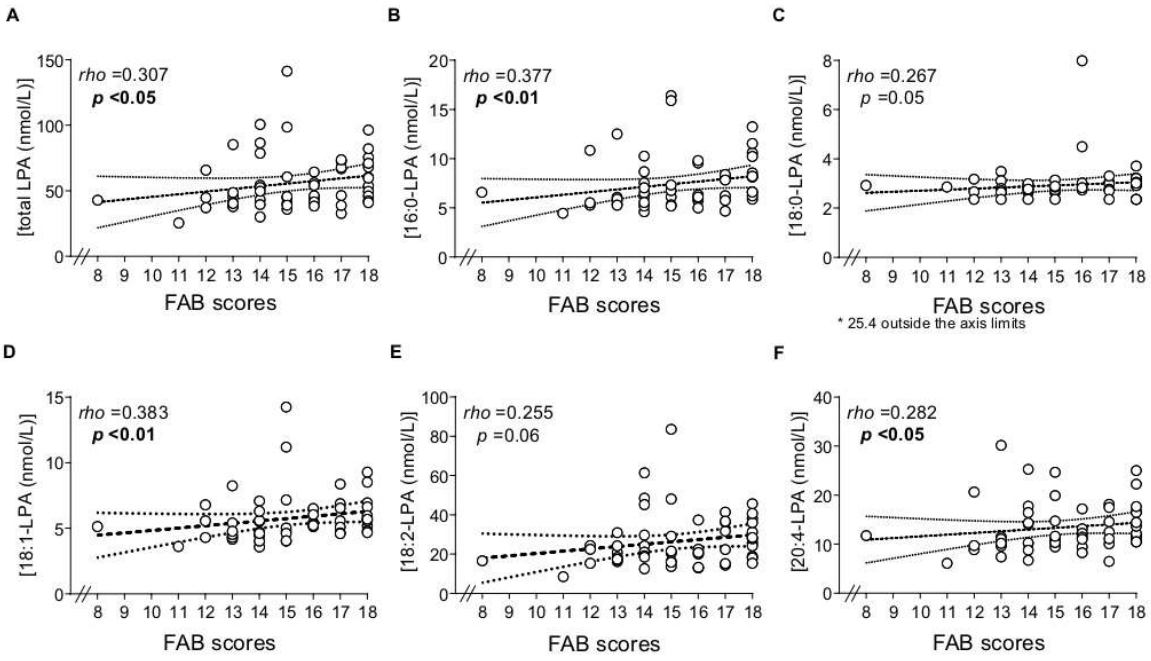

Figure 2. Correlations between LPA species and FAB scores in AUD group controlled by age and sex. (**A**) Total LPA (nmol/L) with FAB scores; (**B**) 16:0-LPA (nmol/L) with FAB scores; (**C**) 18:0-LPA (nmol/L) with FAB scores; (**D**) 18:1-LPA (nmol/L) with FAB scores; (**E**) 18:2-LPA (nmol/L) with FAB scores; (**F**) 20:4-LPA (nmol/L) with FAB scores. Dots are individual values. (rho) Spearman's correlation coefficient; (p) p value for statistical significance.

assessed by MFE scale and in the frontal lobe assessed by FAB scale, in plasma concentrations of total LPA and LPA species in the AUD group.

The AUD group showed a MFE score mean of 29.25 ± 16.47, indicating no deficits related in memory impairment. Moreover, a FAB score mean in AUD group was 15.25 ± 2.23, indicating a mild deficit in frontal lobe assessment (dysexecutive syndrome). Correlation analyses using Spearman correlation (rho) were performed in plasma concentrations of total LPA and LPA species with MFE and FAB scores, respectively (see Supplementary Table S6). In addition, a negative correlation was found between the MFE scores and FAB scores FAB scores ($r = -0.493$, $p < 0.001$).

As shown in Fig. 2, there were significant and positive correlations between the executive tasks evaluated with FAB scores and the plasma concentrations of total LPA, and the plasma concentrations of 16:0-LPA, 18:1-LPA and 20:4-LPA species. It is important to note that all the correlation analyses were double checked using a bootstrapped approach technique, and the Spearman correlation between total FAB and 18:0-LPA was not robust enough to be taken as a significant correlation result. Despite this fact, there were positive correlations between the executive tasks and other LPA species determined except for 18:0-LPA and 18:2-LPA. By contrast, we found no associations between memory impairments assessed with MFE and plasma concentrations of LPA species in the alcohol group. These data suggest a significant association between executive functions and circulating LPA species in the AUD group.

Correlation of plasma concentrations of LPA species with growth factors. Moreover, correlation analyses using Pearson correlation (r) were performed with the logarithms of plasma concentrations of LPA (total LPA and LPA species) and growth factors (BDNF, IGF-1 and IGF-2, and NT-3) in the AUD group (Supplementary Table S7).

As shown in Fig. 3, the statistical analyses found positive and significant correlations between plasma concentrations of BDNF and total LPA, 18:2-LPA and 20:4-LPA. However, there were no significant correlations between BDNF and 16:0-LPA, 18:0-LPA or 18:1-LPA.

Unlike BDNF, there were negative significant correlations between plasma concentrations of IGF-1 and IGF-2 plasma concentrations with some LPA species (Fig. 4). Specifically, IGF-1 correlated negatively with total LPA and all LPA species, except for 18:0-LPA; while IGF-2 only correlated negatively with 18:2-LPA.

Discussion

Preclinical studies in animal models of alcohol dependence corroborate the participation of LPA and its LPA1 receptor in the spontaneous alcohol preference and alcohol drinking of mice, as well as in alcohol-associated changes in emotional memory and social/maternal behavior[34,42]. Another preclinical study using ethanol fed

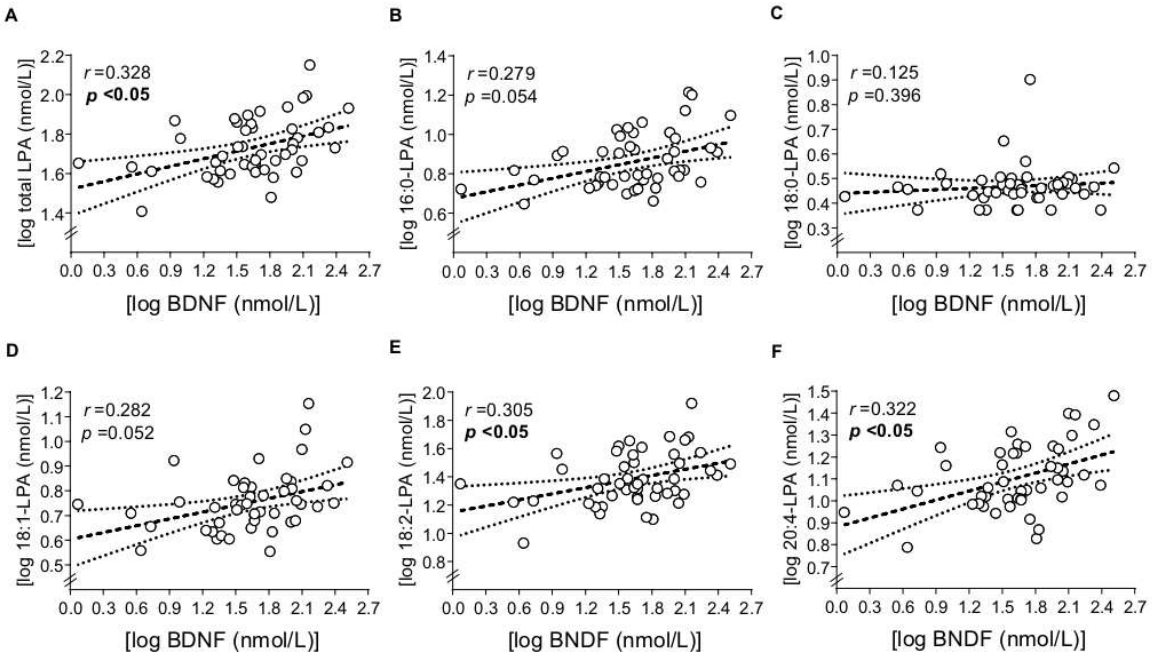

Figure 3. Correlations between LPA species and plasma concentrations of BDNF in AUD patients. (**A**) log BDNF (nmol/L) with log total LPA (nmol/L); (**B**) log BDNF (nmol/L) with log 16:0-LPA (nmol/L); (**C**) log BDNF (nmol/L) with log 18:0-LPA (nmol/L); (**D**) log 18:1-LPA (nmol/L) with log BDNF (nmol/L); (**E**) log BDNF (nmol/L) with log 18:2-LPA (nmol/L); (**F**) log BDNF (nmol/L) with log 20:4-LPA (nmol/L). Dots are individual values. (r) Pearson's correlation coefficient; (p) p value for statistical significance.

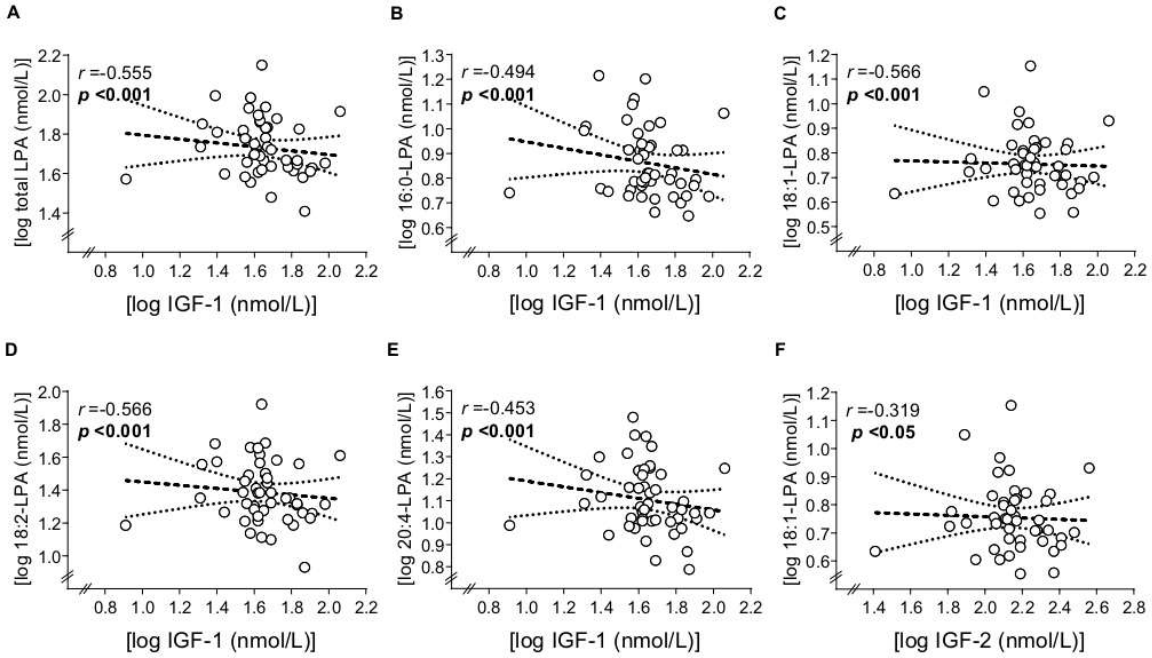

Figure 4. Significant correlations between LPA species and plasma concentrations of IGF-1 in AUD patients. (**A**) log IGF-1 (nmol/L) log with total LPA (nmol/L); (**B**) log IGF-1 (nmol/L) with log 16:0-LPA (nmol/L); (**C**) log IGF-1 (nmol/L) with log 18:1-LPA (nmol/L); (**D**) log IGF-1 (nmol/L) with log 18:2-LPA (nmol/L); (**E**) log IGF-1 (nmol/L) with log 20:4-LPA (nmol/L); (**F**) log IGF-2 (nmol/L) with log 18:1-LPA (nmol/L). Dots are individual values. (r) Pearson's correlation coefficient; (p) p value for statistical significance.

mice has described specific alterations on some fatty acids-related lipids[43]. These results are in agreement with the present study in AUD patients. Thus, our results showed that abstinent AUD patients displayed lower circulating levels of total LPA, 18:0-LPA and 18:1-LPA when compared with a control group. In addition to the present results, other studies have reported a deregulation of circulating fatty acids in AUD patients[41,44], suggesting that fatty acid metabolism including other fatty-acid derived signals might be affected after alcohol exposure. Thus, it has been described higher concentrations of oleic acid in a group of patients with alcohol dependence that in the control group[44]. Following this rationale, we have recently described that AUD patients have elevated plasma concentrations of SEA and OEA, the stearic acid and oleic acid-derived acylethanolamides respectively involved in energy homeostasis and alcohol consumption[41,45]. Interestingly, we observed that total LPA and some LPA species, including 16:0-LPA, 18:2-LPA and 20:4-LPA, were affected by sex. Our results are in accordance with previous studies in healthy subjects that have reported sex differences with increased LPA concentrations in women[30,35]. However, we found differences in LPA concentrations between men and women in the control and AUD groups, suggesting that the presence of a sexual dimorphism in the circulating species of this lipid is not a specific factor associated to the impact of alcohol consumption.

Because our AUD sample displayed a high prevalence of SUDs (41.8%) and mental disorders (63.6%), we also evaluated the existence of a possible association between circulating LPA levels and the presence of SUDs and/or comorbid mental disorders. However, we did not find any significant association, maybe due to the small sample size. The elevated lifetime in psychiatric comorbid disorders in our sample is consistent with other reports in AUD populations[46,47]. Although we did not find significant differences in LPA plasma profiles between AUD patients with and without comorbid disorders, there is a growing body of evidence about the implication of fatty acids in certain mental disorders. Thus, serum levels of 16:0-LPA are upregulated in patients with major depressive disorders compared with a control group[48]. Other study show reduced plasma levels of arachidonic acid in bipolar depression patients[49] and fatty acid deficiency in postmortem brain tissues samples[50]. In addition, the role of LPA species in the etiology of several neuropsychiatric disorders through the LPA1 receptor has also been examined both in human and preclinical models. The LPA deregulation has been studied in complex disorder such as schizophrenia in preclinical models[31,51] and schizophrenia patients[52]. Finally, ATX as the primary biological source of LPA, represents a high-value psychiatric condition target[53]. ATX has been described as a possible biomarker of patients with major depression disorder diagnosis, since the serum levels of this enzyme are reduced in depressive patients compared with healthy controls[54].

However, we found a clear association between LPA plasma concentrations and mild cognitive impairment. In our study, the AUD group displayed a mild deficit in tasks related to executive functions according with alcohol-related cognitive impairments. According to our results, executive functions are particularly affected in AUD population, although there are other neuropsychological processes including memory, emotional and psychosocial skills, visuospatial cognition and psychomotor impaired functions altered in alcohol dependence patients[55,56]. Other studies have reported increased difficulties in motivational processes in addiction treatment patients causing an underestimated impact on the efficacy and management on these clinical treatments[14,57]. Although there were no significant correlations between LPA plasma concentrations and memory impairments in our results, the negative correlation found between lobe function and memory scores, is consistent with early reports suggesting that general memory dysfunctions are related with other types of memory and to executive performance[58]. For that reason, it is of great interest the correlation found between 16:0-LPA, 18:0-LPA, 18:1-LPA and 20:4-LPA and the scores obtained in executive function assessment test. These results suggest that some LPA species might be good reliable markers for the detection of executive dysfunction associated with AUD. These findings support previous studies that have described a relation between dysfunctional levels of LPA signaling and neuropsychological impairments. For example, it has been reported that the plasma levels of LPA negatively correlated with mild cognitive impairments assessed with MoCA test in diabetic patients[59]. Regarding preclinical models, the lack of LPA1 receptor has been associated to cognitive alterations, using spatial memory tasks[60,61]. Moreover, a molecular study focused on lysophosphatidic acid acyltransferases (LPAATs) group of enzymes involved in the production of phosphatidic acid from LPA, shows that the inhibition of lipid metabolism is associated with physiological consequences such as cognitive dysfunction[62].

In the present study, we also evaluated the correlation between the LPA species and growth factors. We found a positive correlation between total plasma concentrations of LPA (or that of polyunsaturated LPA) with circulating levels of the trophic factor BNDF, suggesting that both polyunsaturated LPA species and BDNF might contribute to normal cognitive processing. There is a clear association between the decrease in the circulating levels of these mediators, the impairment of cortical/hippocampal LPA and BDNF signaling, and alcohol associated cognitive impairment[18,19,29,42]. However, the mechanisms of this association are unknown. A potential interesting mechanism might be associated with neuroinflammatory processes. In this regard, previous studies with polyunsaturated fatty acids (i.e. arachidonic acid and their metabolites) have demonstrated a tight association between their ability to modulate both inflammation and BDNF production[63].

Finally, we found that plasma levels of IGF-1 correlated negatively with total LPA, 16:0-LPA, 18:1-LPA, 18:2-LPA and 20:4-LPA and IGF-2 correlated negatively with 18:1-LPA. Previous studies have reported that insulin-like growth factors (i.e., IGF-1 and IGF-2) are associated with the maintenance of the cognitive functioning specially in attention and executive functions[64,65]. However since these growth factors are also decreased by alcohol, but they do not correlate with cognitive impairment or with comorbid mental disorders[17], it is difficult to determine the nature of this association. One possible explanation might be derived of the shift on liver metabolism imposed by alcohol. Alcohol-associated cognitive impairment in the IGF-1 signaling could be a potential mechanism in the neuroinflammatory processes.

Limitations of the present study. These finding described an important effect of alcohol consumption on LPA plasma concentrations, as well as an important association with executive functions and cognitive impairment. Moreover, we are aware about the existence of limitations in the present observational study. First, the recruitment of the sample was conducted from outpatient programs and there are uncontrolled social and environmental variables (e.g., diet, medication control) that could affect the validity of the results. Second, larger samples of male and female AUD patients and additional experimental groups should be included (e.g., patients with mental disorders but without substance use disorders for analyzing in depth the contribution of LPA to alcohol-induced brain damage). Third, longitudinal studies are also needed to monitor changes in these metabolites during abstinence at different times in the same patients. Finally, because a high percentage of AUD patients were under different pharmacological treatment, we cannot exclude the influence of specific medications on the circulating concentrations of the different LPA species.

Conclusions

In agreement with previous preclinical studies supporting a role of the fatty acid related lipids and the lysophosphatidic acid receptor 1 (LPA1) in alcohol consumption[34,42,43], the present results further suggest that LPA species are lipid mediators associated with AUD. The main findings of this study indicate that (a) The total circulating concentration of LPA, 16:0-LPA, 18:0-LPA and 18:1-LPA were decreased in the AUD group when compared to the control group; (b) The total circulating concentration of LPA, 16:0-LPA, 18:2-LPA and 20:4-LPA were decreased in men compared to women (c) The plasma concentrations of the LPA species were not significantly affected by the presence of lifetime comorbid mood and anxiety disorders; (d) In the AUD group, 22% of the patients had cognitive deficits related to executive functions, while 32.6% displayed deficits related to memory impairments; (e) The executive tasks scores of the AUD group correlated with plasma concentrations of total LPA, 16:0-LPA, 18:1-LPA and 20:4-LPA; (f) There is a clear positive correlation between plasma concentrations of BDNF and total LPA, 18:2-LPA and 20:4-LPA; (g) There is a strong inverse correlation between IGF-1 and total LPA, 16:0-LPA, 18:1-LPA, 18:2-LPA and 20:4-LPA. Overall, these data suggest that LPA species are affected by chronic alcohol consumption, and they are associated with cognitive impairments similar to trophic factors such as BDNF[18].

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Author contributions
R.D.T., F.R.F. and A.S. were responsible for the study concept and design. N.G.M. and F.J.P. performed the statistical analysis and the interpretation of findings. N.G.M., N.P., A.S. and F.R.F. drafted the manuscript. M.F.L., N.R.O., P.A. and D.S.P. coordinated the recruitment of the participants in the study and contributed to the acquisition of psychiatric and neuropsychological data by means of the interviews and generated the database. N.P., M.M. and R.D.T. supervised and performed the quantification of LPA species in the human samples; F.J.P. and J.S. supervised and performed the quantification of the growth factors in the human samples. L.S., F.R.F. and A.S. provide critical revision of the manuscript from important intellectual content. All authors critically reviewed the content and approved the final version for publication.

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Competing interests
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Additional information
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El deterioro cognitivo y psiquiátrico subagudo en covid-19 grave.

“The Cognitive and Psychiatric Subacute Impairment in severe Covid-19.”

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Research Article

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Abstract

Background: Neurologic impairment persisting months after acute severe SARS-CoV-2 infection has been described because of several pathogenic mechanisms, including persistent systemic inflammation. The objective of this study is to analyze the selective involvement of the different cognitive domains, it impacts on quality of life and the possible existence of related biomarkers.

Methods: Cross-sectional study of patients who survived severe infection with SARS-CoV-2 consecutively recruited from 13 neurology services in Spain between 90 and 120 days after hospital discharge. All patients underwent an exhaustive study of cognitive functions as well as plasma determination of pro-inflammatory factors (chemokines), and neurotrophic factors and light-chain neurofilaments. A Principal Component Analysis extracted the main independent characteristics of the syndrome.

Results: 152 patients were recruited. The results of our study show a pattern of cognitive impairment with preferential involvement of episodic and working memory, executive functions, and attention and relatively less affectation of information processing speed, denomination, verbal fluency, and other cortical functions. In addition, psychiatric affectation such as anxiety and depression pictures are constant in our cohort. Several plasma chemokines concentrations were elevated compared with both, a non-SARS-Cov2 infected cohort of neurological outpatients or a control healthy general population, suggesting a pro-inflammatory chronic state derived of viral infection.

Conclusion: The neurologic Subacute Impairment in severe Covid-19 consist in an *amnesic and dysexecutive syndrome* with neuropsychiatric manifestations. We do not know if the deficits detected can persist in the long term and, in this case, if this can trigger or accelerate the onset of neurodegenerative diseases.

Introduction

In December 2019, a new coronavirus emerged as a pathogen in the Chinese city of Wuhan, causing severe acute respiratory syndrome (SARS) of high lethality[1]. SARS-CoV-2 spread rapidly throughout the world, and the WHO declared the disease caused by this global virus a pandemic in March 2020. At the time of writing this article, more than 195 million people worldwide had been infected with SARS-CoV-2, resulting in the death of more than 4,1 million individuals [2].

Neurological impairment in this disease has been proven, both in the acute and subacute phases[3–7]. There are 4 mechanisms by which this neurological dysfunction can occurs: direct viral invasion, indirect effects of peripheral inflammation, peripheral organ dysfunction (lung, kidney, and liver) and cerebrovascular endothelial injury[8,9].

Necropsic studies have proven some neuroinvasive capacity of SARS-CoV-2[7,10,11]. Furthermore, the relationship between viral neuroinvasive infections and neurodegenerative diseases (NDDs) has been described [12–16], with preferent injury to the hippocampus and other regions of the temporal and frontal lobes related to cognition [17, 18].

Secondly, a loss of function of the blood brain barrier (BBB) can occur in situations of persistent systemic inflammation such as that occurring in SARS-Cov2 infection. [19, 20, 21]. In addition, endothelial cells are key to the functional integrity of the BBB, and endothelial injury is a recognized element in the pathophysiology of SARS-CoV-2 infection [22].

Finally, peripheral tissue injury typical of serious infections, such as severe COVID-19 can generate danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs), enough for acting on CNS-specific

receptors, leading to microglial activation and, ultimately, pyroptosis or neuronal death of neuroinflammatory origin [23,24].

Cognitive and neuropsychiatric impairment persisting months after acute SARS-CoV-2 infection has been described [25–29]. However, we lack detailed studies that analyze the selective involvement of the different cognitive domains using specifically designed tests, it impacts on quality of life and the possible existence of related biomarkers. This is the objective of our study on a hospital cohort of survivors of a severe SARS-Cov-2 infection.

Material And Methods

Design: Cross-sectional study of consecutive patients who survived severe infection with SARS-CoV-2; The clinical treatment of the patients was performed according to the routine clinical care based on the criteria of the attending physician of each patient.

Inclusion criteria:

- At least one positive PCR test (oropharyngeal swab) for SARS-CoV-2 infection.
- Respiratory failure with criteria for hospital admission; radiological criteria for lung disease (chest CT scan/X-ray with bilateral ground-glass opacities.
- More than 90 days and less than 120 days since hospital discharge.

Exclusion criteria:

- Cognitive impairment with a global deterioration scale (GDS) score of 4 or higher.
- Motor, sensorial, or intellectual disability or illiteracy that prevented performing neuropsychological tests.

Recruitment: Patients were consecutively recruited from 13 neurology services in Spain during the first wave of pandemic through a retrospective review of patients with hospital admission for severe Covid-19. For comparison purposes on circulating plasma chemokines and growth factors, plasma samples from two non-SARS-Cov-2 infected control groups were used. The first group was a cohort of neurological patients (n=46, mean age 71 y. o., SD 10.1, 17 males and 29 females) 60% affected with mild cognitive impairment (Mean MOCA score 18.5, SD 7.6). The second control group was a healthy general population from the National ADN biobank of Salamanca (n=40, mean age 52,2 y.o., SD 2.3, 20 males and 20 females). Both groups were recruited for a different project and its use was approved by the ethical committee of Regional University Hospital of Málaga (RUHM).

Study variables:

Retrospective Data collected during admission period:

- **Clinical**: Age, sex, length of hospital stays, comorbidities, symptoms related to SARS, neurological symptoms during admission and anti-COVID drug treatment.
- **Analytical**: Complete blood count, serum electrolytes, total protein, C-reactive protein, D-dimer, creatine kinase (CK), lactate dehydrogenase (LDH), transaminases, blood urea nitrogen (BUN), creatinine and ferritin.

Data collected during the visit (90-120 days after hospital discharge):

- **Neuropsychological study protocol:**

Global Cognition was studied through the Montreal Cognitive Assessment (MoCA) for dementia.

Memory was evaluated using the Spanish version of the California Verbal Learning Test (CVLT) also named Test de Aprendizaje Verbal España-Complutense (TAVEC) and Free and Cued Selective Reminding Test (FCSRT) for verbal episodic memory, Boston Naming Test (BNT) for denomination capacity, Rey Complex Figure Test (RCFT) for visuospatial episodic memory, and Digit Retention Test (DRT) of Wechsler Adult Intelligence Scale (WAIS) for working memory and memory reserve.

Executive function (RCFT, Trail Making Test Time B (TMT-B) and the Verbal Fluency Test (FAS)) and attention (TMT-A), were also evaluated.

Psychiatric impairment was evaluated using the State-Trait Anxiety Inventory (STAI) and Beck Depression Inventory II (BDI-II) tests.

All tests have been previously standardized for age, sex and educational level for the Spanish population. Specifically, FCSRT, BNT, RCFT, FAS, DRT (verbal span) and TMT-A, and B were standardized within the framework of the NEURONORMA project [30–33]. The rest of the tests have been standardized for age, sex and education level when possible according with their respective published normative values: TAVEC[34], MoCA[35], STAI[36] and BDI[37].

An estimate of quality of life was made with the EuroQol 5D test (EQ-5D), also validated for Spanish population[38].

- **Analytical Study Protocol:**
    - **Basic screening**: The same as that collected during admission.
    - **Plasma chemokines and growth factors**: MIP-1alpha/CCL3 (Macrophage inflammatory proteine-1-alpha), SDF-1 (Stromal cell-derived factor 1), Fractalkine/CX3CL1, Eotaxin 1, BDNF (Brain derived neurotrophic factor), VEGF (Vascular Endothelial Growth Factor) and MCP-1/CCL2 (Monocyte Chemotactic Protein-1) levels were determined using the Luminex™ xMAP technology platform. All samples, control groups and COVID patients were measured in the same plates for avoiding interassay variability.
    - **Serum neurofilaments**: Light Chain Neurofilament (NFL) levels were determined using a digital enzyme immunoassay and the SIMOA HD1 Analyser platform.

Interpretation of the tests: To reduce the impact of the absence of a cognitive function study prior to infection on the correct interpretation of our results, we have used tests with normative values for the Spanish population. To categorize each patient individual result in normal or abnormal values we used cut-off points stratified by age, sex and educative level. Abnormal values were defined by ±1 SD (±1Z) of the mean for the reference group of age, sex and educational level. This criterion was chosen instead of others, such as ±1.5 SD, to increase the sensitivity of the deviation from the mean when categorizing the test results; we did not intend to categorize the results as “healthy” or “pathological” but simply detect deviations from normal state.

See the supplementary material for a list of the tests (Supplementary Table S1) and for an explanation of the protocol used for neuropsychological evaluations (Supplementary Box 1).

Statistical study: All clinical data, laboratory variables and diagnostic tests were entered into a database for analysis using statistical software IBM SPSS version 21.0. For numerical data, the normality of the distribution of the data was determined by the Kolmogorov-Smirnov test. Data calculated as percentages were analysed using the chi-squared test. For the data expressed as the mean ± standard deviation, Student's T test or the Mann-Whitney/Kruskal Wallis test were used depending on the normality of the sample.

To determine the main components of our database, principal components analysis (PCA) was used; the analysis included related quantitative variables as well as age, length of hospital stay, and the analytical parameters found to be pathological during hospital admission. The Bartlett sphericity test and the Kayser-Meyer-Olkin (KMO) sample adequacy test were applied to demonstrate the adequacy of this type of analysis for our sample.

Correlations of the isolated components were analysed using Pearson's R for continuous and normally distributed data and using Spearman's rho for nonnormally distributed data.

Last, linear regression analysis of isolated components was performed using the score on the EQ5 quality of life scale as the dependent variable.

The missing data were excluded from statistical analysis except in the PCA, in which they were replaced by the mean.

Ethical considerations: This study was approved by the Ethics and Clinical Research Committee of the RUHM. Each participant or legal representative signed an informed consent form after receiving a complete description of the study and being given the opportunity to ask any questions. The process of obtaining informed consent adhered to the principles of the Declaration of Helsinki of the World Medical Association.

Results

A total of 152 patients infected with SARS-CoV-2 who met all inclusion criteria and none of the exclusion criteria were recruited. The group was composed of 46 patients with long-term depressive symptoms, 25 with a history of stroke (16 territorial and 9 lacunar), 11 with chronic anxiety symptoms, 6 with Parkinson's disease, 6 with subjective memory failure (with GDS <4), 3 with Multiple Sclerosis, 3 with non-lesional focal epilepsy, 1 with Guillain-Barré syndrome and 11 with another chronic neurologic conditions without dementia. In this sense, we must consider that our cohort is composed mainly of patients with neurological or psychiatric vulnerability but without cognitive impairment, as required by the inclusion criteria.

The epidemiological data, symptoms during admission and specific treatments received during admission are provided in Table 1.

The means for the analytical variables assessed during admission were within the normal range of our laboratory, except the following:

- D-dimer: mean value, 1266.02 ng/ml (SD: 1969);
- Ferritin: mean value, 703.53 mcg/l (SD: 662.22); and
- C-reactive protein (CRP): mean value, 94.49 mg/l (SD: 85.45).

Compared with the values obtained during the study visit (90-120 days after hospital discharge), the ferritin values were found in the normal range for our laboratory**,** while the D-dimer (586.36 ng/ml; SD = 683.75) and CRP (6.47 mg/ml; SD = 16.15) values remained slightly elevated, although their values had decreased substantially.

All analytical test results are available as supplementary material to this article (Supplementary Table S2).

Table 2 provides the psychopathological evaluation data for the sample.

Table 3 provides the total MoCA test score as well as the scores for the 7 subdomains.

Table 4 provides the results of the cognitive exhaustive evaluation test used for the sample.

Figure 1 provides the data for chemokines and growth factors in general population, mild cognitive impairment control group and COVID patients.

Supplementary table S3 provides the numerical data for chemokines, growth factors and NFL.

For a graphical representation of the cognitive deficits detected in our sample the results are presented as a function of the percentage of the maximum score obtained on each test and as the total score (Figure 2).

Principal components analysis:

To reduce the number of variables, we performed PCA, in which we included the following quantitative variables: age, length of stay, pathological analytical variables during admission (ferritin and D-dimer) and numerical variables corresponding to the cognitive and neuropsychiatric test results. Six components capable of explaining 55.34% of the variance were identified. The KMO value was 0.854, and the Bartlett sphericity test indicated a significance of <0.0001, confirming the power and adequacy of the analysis.

The rotated components matrix and the explained variance table are provided as supplementary material (Supplementary Tables S4 and S5, respectively).

Correlation with quality of life:

A regression analysis of the identified components was performed using quality of life as the dependent variable; the results are provided in Supplementary Tables S6, S7 and S8). A Durbin-Watson test value less than 2 ensures that the factors are not autocorrelated. The result of the analysis showed that components 2 (global cognition/executive functions) and 6 (impairment of the neuropsychiatric area) explained the variable quality of life with high significance.

Plasma concentration of proinflammatory chemokines and growth factors (Figure 1):

We selected five chemokines and two growth factors that have been related to neuroinflammation and cognitive impairment/neurodegeneration previously. Kruskal-Wallis analysis show that the chemokines SDF-1a ($H=7.3$. $p<0.001$), MCP-1 ($H=14.1$ $p<0.01$) and Eotaxin-1 ($H=37.5$. $p<0.001$) were elevated in post-Covid patients, as well as the trophic factor BDNF ($H=28.7$. $p<0.001$), when compared with both control groups. In addition, Fractalkine ($H=14.0$. $p<0.01$), and VEGF-A ($H=11.1$. $p<0.01$) were elevated when compared only with the MCI cohort. MIP1-A was equal among groups ($H=4,9$ $p=0.1$, nonsignificant). These results suggest a pro-inflammatory chronic stated derived of severe COVID-19 disease. Remarkably, the circulating pattern of chemokines and growth factors in postcovid patients was found to be the opposite of that of non-infected age-matched patients attending the neurology department because of subjective memory deficits complaints (MCI cohort).

Correlation with plasma proinflammatory factors and NFL:

To identify plasma markers that could potentially be related to the main components of this Syndrome, a bivariate correlation analysis was performed using components 1 and 4 and the values obtained for each given plasma factor. The results are shown in Table 5.

Discussion

The results of our study show a pattern of cognitive impairment with some peculiarities. Thus, there is preferential involvement of episodic memory, working memory, executive functions, attention, and relatively less affectation of information processing speed, denomination, verbal fluency, and other cortical functions such as visuo-constructive

ability (Table 4; Figure 2). In addition, the detection of psychiatric affectation such as anxiety and depression pictures are constant in our cohort (Table 2). So, we could therefore refer to post-covid syndrome as an amnesic and dysexecutive syndrome with impaired attention and psychiatric comorbidity. This pattern can be typified as suggestive of fronto-subcortical involvement and can be found in other situations with a neuroinflammatory basis of viral ethiology (eg, HIV encephalopathy)[17].

After PCA, the following 6 independent components were identified as the cognitive and psychopathological areas affected.

Components 1 and 5: These 2 components are grouped because they integrate **variables related to episodic memory**, that is, memory related to vital events. Component 1 includes some scores of the TAVEC test as well as the free memory score of the FCSRT test, which primarily evaluates episodic verbal memory. Furthermore, Component 5 includes the other scores for the FCSRT test (cued and delayed recall).

Impairment in ***episodic verbal memory*** was observed in 34.7% (for TAVEC short term free memory) to 38.5% (for TAVEC Recall with long-term keys) of our patients and constitutes a specific element of this syndrome. Additionally**, *working memory*** measured through the Digit Retention Test (DRT) (WAIS-IV), was affected in 26.4%-36.7% of the sample. Other types of memory such as ***semantic memory,*** explored through the BNT seem less affected.

Traditionally, this type of mnemonic impairment has been related to subcortical cognitive impairment and has been identified in other neuroinflammatory processes of viral origin, such as HIV-associated neurocognitive disorders (HANDs) [39].

Component 2: Variables related to global cognitive function and visuo-spatial abilities: the overall MoCA score and subdomain scores, except for orientation and animal naming (which are integrated in component 4), and the FCSR direct copy and memory scores, which both measure visuospatial and executive function.

The mean overall MoCA score in our sample was 21.95 (±5.70) points. Deficits were identified by low scores in delayed recall and, to a lesser extent, in attention, abstraction and language.

In general, the scores of the tests included in this component was not very deficient. Our patients are preferably included in the spectrum of mild cognitive impairment (MCI) with relative respect for purely cortical functions such as visuospatial function.

Component 3: Component 3 includes variables related to **executive functions** and **verbal fluency.** The impairment of ***executive functions*** was substantial in our patients as shown by the scores of the FAS animals (43.7% abnormal), FAS vegetables (48.6% abnormal) and FAS kitchen (33.1% abnormal) tests, more related to executive functions. We attribute this result to a failure in executive functions reinforcing the idea that frontal lobe dysfunction is frequent in post-Covid19 syndrome.

These findings are consistent with published functional impairment data, especially in studies that evaluated brain positron emission tomography with fluorodeoxyglucose (FDG-PET) and demonstrated greater impairment in the amygdala, hippocampus, parahippocampal region and frontal lobes [40–42], areas directly related to memory and executive functions.

Component 4: The component includes **variables related to attention**, especially TMTs A, and **orientation and naming** (subtests of the MoCA). The scores obtained were abnormal for the 34.2% of the sample for the TMTs A. This finding

is commonly described in patients with inflammatory-based encephalopathies [29,43]. The two MoCA subdomains included in this component that were less affected: Animal naming and Orientation.

Component 6: Depression and Anxiety related variables.

Over the course of the pandemic, the general population has been subjected to stressors derived from the social and economic impacts of the virus [28,44]. It is, for this reason, difficult to separate the actual contribution of the biological factors highlighted in this article from other environmental factors. The BDI-II scores for our sample correspond to mild depression. Overall, 27.4% of patients had a BDI score equal to or greater than 20 points, which indicates a clinical diagnosis of moderate or severe depression [45]. A total of 35.56% of the total sample had state STAI-State scores compatible with state anxiety. The results of our study show that regardless of its origin, neuropsychiatric impairment is another of the essential elements of the syndrome.

Cognitive impairment associated with depression is clearly seen in severe depression[46]. However, the depression that we see in our patients is mild (mean score on the BDI-II = 14.95). For this reason, the non-existence of correlation with cognitive variables is not surprising.

Integrating all these results, we can state that the subacute neurological impairment in severe Covid-19 can be defined as a *global cerebral condition in the spectrum of MCI, characterized by a predominant deterioration of memory (especially, episodic and working memory), executive functions, attention, and neuropsychiatric impairment.*

Our results identify a pattern of subcortical deterioration with similarities to that described in cerebral small-vessel diseases with the predominant endothelial injury [47,48]. There are also semiological parallels with neuroinflammation-based encephalopathies [26,27,29]. Among the plasma factors studied herein, some, such as CRP and NFL, are correlated with endothelial injury [49].

Although our cohort is quite homogeneous, it could be hypothesized whether there could be different manifestations based on the previous existence of a situation of greater cognitive or psychiatric vulnerability. To test this possibility, we performed a post-hoc analysis dividing the sample into patients with a history of neurological or psychiatric disease versus those who did not. The results showed that there were no differences between these subgroups, thus highlighting the homogeneous nature of our results. We present these data as supplementary material (Tables S9-S12).

Quality of life was directly correlated with the main components that measure global cognitive function, and neuropsychiatric impairment; in the latter case, there was an inverse correlation with high trait anxiety and state anxiety scores and depression. We propose that impairment in these domains most determine the quality of life of our patients.

Interestingly, the analysis of circulating chemokines and growth factors suggest that 3 months after discharge, COVID-19 patients have a persistent neuroinflammatory state. This activation appears to be derived only of the infection by SARS-Cov-2, and not being associated to age-associated cognitive impairment, since patients with MCI not infected displayed a clearly different set of plasma chemokine concentrations.

The correlation analysis of the components with plasma chemokine proinflammatory factors found few significant correlations.

So, there was an inverse correlation between NFL levels and the Components related to the measurement of episodic memory (Rho=-0.310; p=0.018), global cognition (Rho=-0.297; p=0.024) and to Executive functions (Rho=-0,417;

p=0.001). NFL are markers of neuronal destruction whose correlation with global cognition has been described in the literature [50]. Plasma NFL levels could be a robust biomarker of this syndrome.

Vascular Endothelial Growth Factor (VEGF) showed inverse correlation with the variables included in Component 4, mainly related to Attention. VEGF has been linked to endothelial dysfunction which, as already mentioned, appears to be an element present in SARS-Cov2 infection [8] and more specifically, with cognitive decline present in some diseases with a large vascular component, such as DM [51]. This finding reinforces our hypothesis that Post-Covid Neurologic Syndrome is intimately related to the typical vascular damage of Covid-19 disease.

In conclusion this Syndrome is a distinct condition that persists for at least 12 weeks after overcoming the acute phase of SARS-CoV-2 infection. The profile remains stable in different stratified populations based on cognitive vulnerability. Last, we identified biomarkers related to the main components of the syndrome. The possibility of any of them behaving as a prognostic biomarker and even as possible future therapeutic strategy development for Post-Covid Neurologic Syndrome should not be ruled out.

The main limitation of our study is the absence of an assessment of cognitive and neuropsychiatric function and of plasma markers prior to infection, preventing us from reliably measuring the impact of the infection. To minimize the impact of this fact on the interpretation of our results, we have used tests that have normalized values for the Spanish population, so that we can consider that the real control group in this study is the own general population stratified by age, sex and educational level.

Some issues remain to be resolved. First, we do not know whether the deficits detected are transitory or persist long term. If long term, it is unknown whether the underlying neuroinflammatory phenomena can trigger NDDs in a manner analogous to what probably occurred during the 1918 “Spanish flu” pandemic [16].

Declarations

Data Sharing and Data Accessibility: All databases used during the preparation of this article are available to editor or reviewers if required and have been incorporated into the DRYAD repertoire (Dryad Home - Publish and Preserve your Data (datadryad.org)).

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Appendix

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Tables

Epidemiological characteristics		
Sex (V. %)		70/152 (46.1%)
Age in Years (±SD)		69.09 (±11.37)
Duration of admission in days) (±SD)		14.38 (±13.14)
Ethnicity	Caucasian	150 (98.7%)
	Latin	2 (1.3%)
Level of study	Primary education	73 (48.0%)
	Secondary education	34 (22.3%)
	Higher education	21 (13.8%)
	No studies	15 (9.8%)
	Unknown	9 (5.9%)
Marital status	As a couple	99 (65.1%)
	Single	9 (5.9%)
	Widower	18 (11.8%)
	Separate	21 (13.8%)
Previous GDS	GDS1	108 (71.0%)
	GDS2	35 (23.0%)
	GDS3	7 (4.6%)
Comorbidities	HT	95 (62.5%)
	DM	39 (25.6%)
	Previous cerebrovascular disease	47 (30.9%)
	Cancer disease	8 (5.2%)
	Smoking	15 (9.8%)
	Regular alcohol consumption	28 (18.4%)
General symptoms during admission		
Fever (Temperature > 38ºC)		123 (80.9%)
Cough		107 (69%)
Dyspnea		90 (58.1%)
Anorexia		47 (30.3%)
Diarrhea		55 (35.5%)
Neurological symptoms during admission		
Headache		34 (21.9%)
Loss of awareness		11 (7.1%)
Seizures		1 (0.6%)
Stroke		2 (1.2%)
PNS involvement		6 (3.9%)
Myalgias		17 (11%)
Anti-Covid19 treatments		
Hydroxychloroquine		112 (72.3%)
Lopinavir/Ritonavir		69 (44.5%)
Others (Methylprednisolone. Anti-IL)		98 (64.4%)

'able 1. Epidemiological and clinical data during admission. DM: Diabetes mellitus. GDS: Gl
)eterioration Scale. HT: Hypertension. PNS: Peripheral Nervous System.

	N	Average Direct Score (±SD)	N (%) ≤1Z
BDI	135	14.95 (±10.73)	**37 (27,40%)**
STAI State	138	23.79 (±10.98)	**49 (35,56%)**
STAI Trait	138	24.18 (±11.18)	**41 (29,49%)**

Table 2. Results of the psychopathological assessment of the cohort.

	N	Average Direct Score (±SD)	Maximum Score	Average Scaled Score	N (%) ≤1Z
Global MoCA score	131	**21.95 (±5.70)**	30	10.86 (±8.85)	**33 (25.2%)**
Visuo-spatial and executive function	129	**3.63 (±1.49)**	5		
Animal naming	129	**2.78 (±0.53)**	3		
Attention	129	**4.23 (±1.66)**	6		
Language	129	**2.07 (±1.10)**	3		
Abstraction	129	**1.33 (±0.70)**	2		
Delayed recall	129	**1.94 (±1.69)**	5		
Orientation	129	**5.62 (±0.92)**	6		

3. MoCA test scores in the global sample. Abnormal values are considered if they are equal to or less t
low the value corresponding to their standardized group by age, sex and educational level.

	N	Average Direct Score (±SD)	Average Scaled Score (±SD)	Z (±SD)	N (%) ≤1Z
ɜC (Verbal Episodic Memory)					
ɜC Learning	118	35.91 (±15.02)		-0.87 (±1.54)	49 (41.5%)
ɜC Short-term free ory	118	8.01 (±5.55)		-0.30 (±1.74)	39 (33.3%)
ɜC Recall with short- keys	118	8.77 (±3.7)		-0.55 (±1.17)	41 (34.7%)
ɜC Long-term free ory	117	7.53 (±4.2)		-0.61 (±1.27)	41 (35.0%)
ɜC Recall with long- keys	117	8.77 (±3.87)		-0.59 (±1.27)	45 (38.5%)
ɜC Recognition	117	13.36 (±3.2)		-0.26 (±1.60)	24 (20.4%)
(Denomination)					
'	141	12.30 (±3.13)		0.06 (±1.37)	24 (17.0%)
ſ (Visuospatial Episodic Memory/ Executive Function)					
ſ Time Copy	123	242.11 (±127.66)	11.67 (±4.72)	0.56 (±1.57)	19 (17.4%)
ſ Copy Direct Score	109	28.73 (±9.42)	10.46 (±3.51)	0.15 (±1.17)	18 (14.7%)
ſ Memory Direct Score	101	11.29 (±8.47)	8.26 (±3.69)	-0.58 (±1.23)	40 (39.6%)
(Attention / Executive Function)					
Time A (Attention)	114	94.96 (±80.83)	7.71 (±3.7)	-0.76 (±1.24)	39 (34.2%)
Errors A	108	0.46 (±1.23)			
Time B (Executive tion)	91	182.25 (±141.33)	8.93 (±3.15)	-0.36 (±1.05)	28 (31.1%)
Errors B	87	1.87			
as Spanish version of FAS (Executive Function* and Verbal fluency**)					
P**	142	10.00 (±5.27)	8.06 (±3.40)	-0.48 (±1.01)	47 (33.1%)
M**	142	8.49 (±4.92)	8.56 (±3.53)	-0.28 (±1.07)	38 (26.8%)
R**	142	8.43 (±4.73)	8.44 (±4.74)	-0.16 (±0.84)	30 (21.1%)

Animals*	141	**13.54 (±5.69)**	7.30 (±3.12)	-0.70 (±0.96)	**62 (43.7%)**
Vegetables*	142	**14.00 (±5.27)**	8.63 (±3.32)	-0.80 (±1.08)	**69 (48.6%)**
Kitchens*	142	**12.26 (±4.45)**	9.99 (±3.61)	-0.39 (±1.19)	**47 (33.1%)**
(WAIS) (Working memory / Memory Reserve)					
s Direct Span	121	**4.95 (±7.54)**	8.11 (±3.64)	-0.63 (±1.21)	**44 (36.7%)**
s Reverse Span	121	**3.25 (±1.38)**	11.32 (±17.26)	-0.05 (±1.15)	**32 (26.4%)**
RT (Verbal Episodic Memory)					
RT Free memory	128	**19.53 (±9.07)**	9.23 (±3.54)	-0.25 (±1.18)	**40 (31.25%)**
RT Cued memory	127	**17.11 (±7.16)**			**32 (26.3%)**
RT Total	127	**35.76 (±11.04)**	10.17 (±4.19)	0.06 (±1.39)	**32 (25.2%)**
RT Delayed	127	**6.74 (±3.95)**	9.10 (±3.54)	-0.30 (±1.28)	**39 (30.7%)**
RT Total Delayed	127	**11.10 (±4.77)**	9.50 (±4.92)	-0.16 (±1.64)	**42 (33.1%)**
ity of life					
D	141	**62.94 (±21.84)**			

4. Complete cognitive evaluation of the cohort.

Measured Factor	N	Component 1 (Episodic memory)	Component 2 (Global Cognition)	Component 3 (Executive Functions)	Component 4 (Attention)	Component 5 (Episodic memory)	Component 6 (Depression and Anxiety disorder)
MIP-1 (CCL3)	106	0,107 (0.276)	0,013 (0.892)	0,048 (0.64)	-0,061 (0.535)	-0,060 (0.538)	0,002 (0.983)
SDF-1 (CXCL12)	117	0,028 (0.763)	-0,053 (0.571)	0,106 (0.254)	-0,148 (0.112)	-0,008 (0.931)	0,072 (0.440)
Fractalkine (CX3CL1)	104	0,008 (0.932)	-0,016 (0.875)	-0,157 (0.111)	***-0,318 (0.001)***	-0,034 (0.735)	-0,115 (0.247)
Eotaxine (CCL11)	120	0,032 (0.729)	-0,032 (0.726)	***-0,226 (0.013)***	-0,064 (0.486)	-0,060 (0.516)	0,022 (0.814)
BDNF	105	0,117 (0.233)	0,050 (0.611)	***-0,213 (0.029)***	-0,024 (0.806)	-0,157 (0.109)	-0,149 (0.129)
VEGF	108	0,050 (0.606)	0,011 (0.910)	***-0,241 (0.012)***	***-0,203 (0.035)***	-0,131 (0.178)	-0,031 (0.754)
MCP-1 (CCL2)	116	-0,005 (0.960)	-0,077 (0.412)	-0,174 (0.062)	-0,102 (0.277)	0,017 (0.855)	-0,128 (0.169)
NFL	58	***-0,310 (0.018)***	***-0.297 (0.024)***	***-0,417 (0.001)***	0,101 (0.452)	-0,049 (0.717)	-0,079 (0.554)

Bivariate correlation between identified components and chemokine levels. In all cases we expressed Sp

Figures

Figure 1

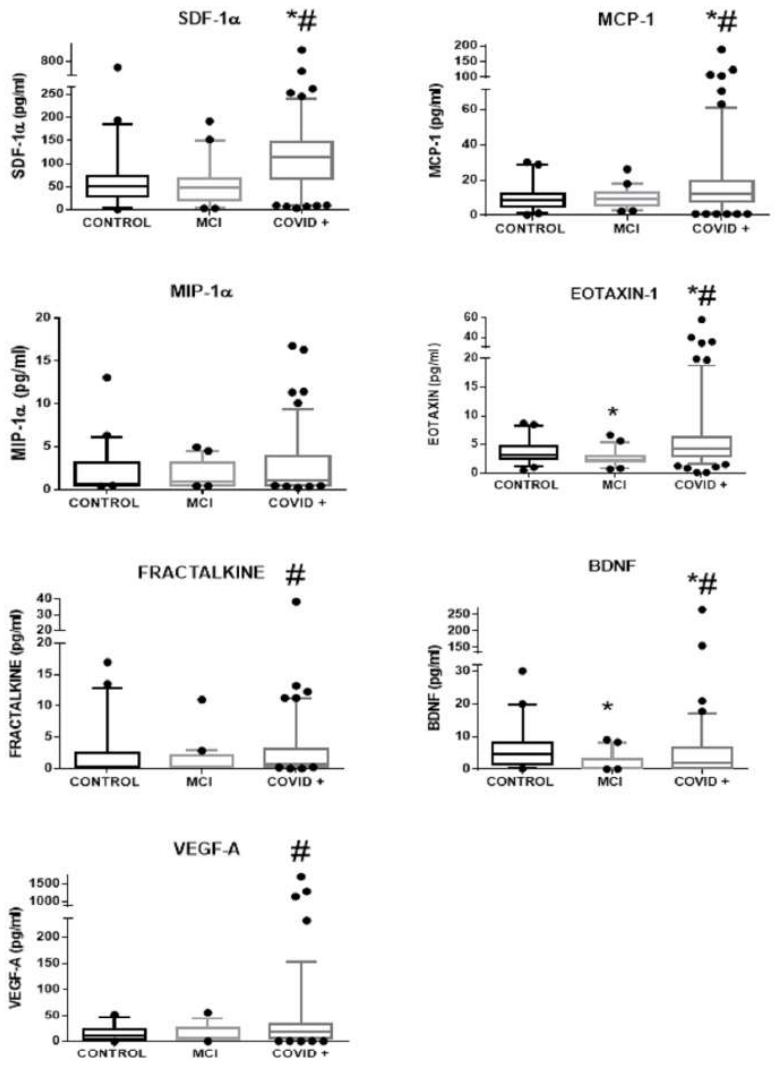

Figure 1

Plasma values of several chemokines and growth factors in control subjects (n=45), mild cognitive impairment patients (MCI, n=41) and COVID-19 patients (COVID+, n=128) 3-4 months after hospital discharge. Kruskal-Wallis Analysis. * P<0.01 versus control group. # p<0.01 versus MCI group. Data in boxplots are means and 5-95 confidence intervals

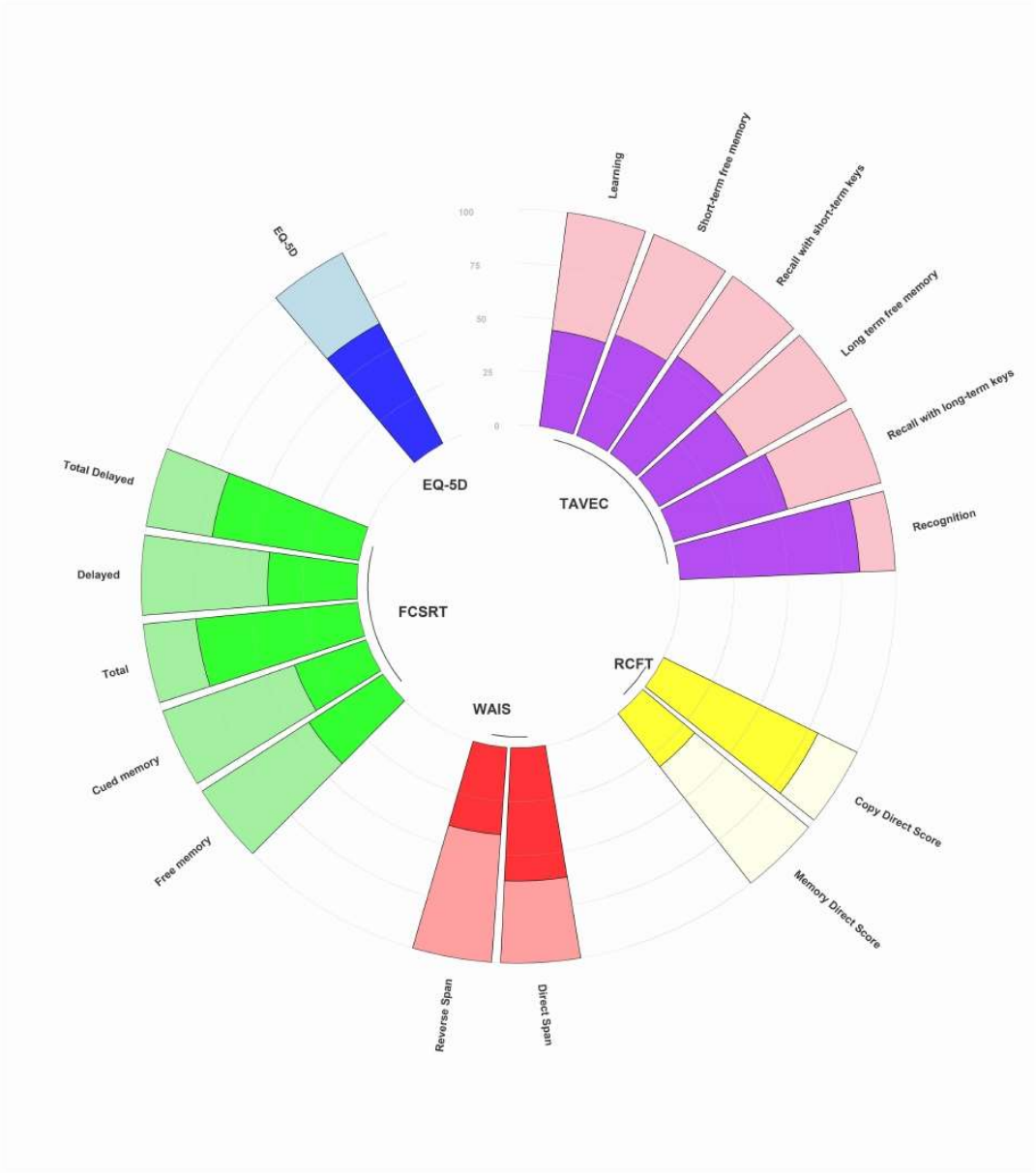

Figure 2

Graphical representation of the profile of the main test used in our study comparing the average vs maximum score.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PaperBrumaSupplementaryMatherialENGRev.docx

La presente tesis ha sido financiada por:

- Proyecto: ALCOHOL, DEPRESSION AND COGNITIVE IMPAIRMENT: THE ROLE OF NON-CANNABINOID ACYLETHANOLAMIDES. Organización de realización: INSTITUTO IBIMA DE MÁLAGA. Principal Investigador: Fernando Rodríguez de Fonseca. Entidad financiadora: Financed by the ISCIII and Co-financed by FEDER Funds. Código según financiador: PI19 / 01577
- Proyecto: CLINICAL-BIOLOGICAL-MOLECULAR CHARACTERIZATION OF NEUROLOGICAL AFFECTION IN COVID-19 DISEASE. IS THERE NEURO-COVID-19 DISEASE? Entidad financiadora: INSTITUTO DE SALUD CARLOS III. Cov20/00157. Principal Investigador: Fernando Rodríguez de Fonseca and Pedro Jesús Serrano Castro. Proyecto: CIRCULATING BIOMARKERS OF PSYCHIATRIC COMORBIDITY IN DRUG USE DISORDER: COMPARISON WITH BIOMARKERS OF DEPRESSIVE DISORDER AND PSYCHOTIC DISORDER. Entidad financiadora: MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD. Principal Investigador: Fernando Rodríguez de Fonseca. Nombre del programa: Research Projects NATIONAL PLAN ON DRUGS. Código según financiador: PND 2018-044.
- Proyecto: RED DE TRASTORNOS ADICTIVOS. Entidad Financiadora: INSTITUTO DE SALUD CARLOS III. Código según financiador: RD16/0017/0001

Presentación de tesis doctoral en formato publicaciones:

Esta Tesis Doctoral se presenta como compendio de publicaciones originales según la normativa aprobada por la Junta de facultad el 17 de abril de 2015 en el desarrollo del Real Decreto 99/2011, de 28 de enero (BOE 10/02/2011) que regula los estudios de doctorado en la UCM (acuerdo de Consejo de Gobierno de 6 de noviembre de 2012, publicado en BOUC 14, de 21/12/2012) que permite la presentación de Tesis Doctorales como compendio de publicaciones.

Este trabajo incluye una introducción general a las publicaciones, un apartado de hipótesis y objetivos en el que se justifica la hipótesis de trabajo y los objetivos de la investigación, una metodología general y específica de los artículos, un apartado de publicaciones en el que se insertan los artículos originales o aceptados para su revisión, una discusión general integradora de las mismas y un apartado con conclusiones específicas y generales.

Para el desarrollo de la presente Tesis Doctoral, en la línea de investigación del **Factores protectores y biomarcadores de deterioro cognitivo en el Trastorno por Uso de Alcohol**, se han realizado los siguientes trabajos de los que han derivado las siguientes publicaciones:

1. Requena-Ocaña, N.; Flores-Lopez, M.; Papaseit, E.; García-Marchena, N.; Ruiz, J.J.; Ortega-Pinazo, J.; Serrano, A.; Pavón-Morón, F.J.; Farré, M.; Suarez, J.; Rodríguez de Fonseca, F.; Araos, P. Vascular Endothelial Growth Factor as a Potential Biomarker of Neuroinflammation and Frontal Cognitive Impairment in Patients with Alcohol Use Disorder. *Biomedicines* 2022, *10*, 947. https://doi.org/10.3390/biomedicines10050947
2. Requena-Ocaña N, Araos P, Flores M, García-Marchena N, Silva-Peña D, Aranda J, Rivera P, Ruiz JJ, Serrano A, Pavón FJ, Suárez J, Rodríguez de Fonseca F. Evaluation of neurotrophic factors and education level as predictors of cognitive

decline in alcohol use disorder. *Scientific Reports*. 2021 Aug 2;11(1):15583. https://doi.org/10.1038/s41598-021-95131-2

3. Requena-Ocaña N, Flores-Lopez M, Martín AS, García-Marchena N, Pedraz M, Ruiz JJ, Serrano A, Suarez J, Pavón FJ, de Fonseca FR, Araos P. Influence of gender and education on cocaine users in an outpatient cohort in Spain. *Scientific Reports*. 2021 Oct 22;11(1):20928. https://doi.org/10.1038/s41598-021-00472-7N
4. Nerea Requena-Ocaña, Pedro Araos, Pedro J. Serrano-Castro, María Flores-López, Nuria García-Marchena, Begoña Oliver-Martos, Juan Jesus Ruiz, Ana Gavito, Francisco, Javier Pavón, Antonia Serrano, Fermin Mayoral, Juan Suarez, Fernando Rodríguez de Fonseca. Plasma concentrations of neurofilament light chain protein and brain-derived neurotrophic factor are consistent biomarkers of cognitive impairment associated to alcohol use disorders. Submitted for review.
5. Nerea Requena-Ocaña; M. Carmen Mañas-Padilla; M. Carmen Pérez-Gómez; María Flores-López; Nuria García-Marchena; Antonia Serrano; Juan Suarez; Francisco Javier Pavón-Morón; Estela Castilla-Ortega; Fernando Rodríguez de Fonsecal, Pedro Araos. Cognitive reserve in substance use disorders and potential biological markers. Submitted for review.

Adicionalmente, y durante el periodo de ejecución de la Tesis, he participado como investigadora activa en las siguientes publicaciones científicas, relacionadas con mi tema de Tesis Doctoral:

1. Flores-López M, García-Marchena N, Araos P, Requena-Ocaña N, Porras-Perales O, Torres-Galván S, Suarez J, Pizarro N, de la Torre R, Rubio G, Ruiz-Ruiz JJ, Rodríguez de Fonseca F, Serrano A, Pavón-Morón FJ. Sex Differences in Plasma Lysophosphatidic Acid Species in Patients with Alcohol and Cocaine Use Disorders. Brain Sci. 2022 Apr 30;12(5):588. doi: 10.3390/brainsci12050588. PMID: 35624975; PMCID: PMC9139721.

2. García-Marchena N, Marcos A, Flores-López M, Moreno-Fernández M, Requena-Ocaña N, Porras-Perales O, Torres-Galván S, Araos P, Serrano A, Muga R, Ruiz-Ruiz JJ, Rodríguez de Fonseca F, Ambrosio E, Pavón-Morón FJ. Plasma Amino Acid Concentrations in Patients with Alcohol and/or Cocaine Use Disorders and Their Association with Psychiatric Comorbidity and Sex. Biomedicines. 2022 May 14;10(5):1137. doi: 10.3390/biomedicines10051137. PMID: 35625874; PMCID: PMC9138967.
3. Flores-López M, García-Marchena N, Pavon FJ, Lara E, Porras-Perales O, Araos P, Requena-Ocaña N, Torres-Galván S, Mañas-Padilla MC, Rubio G, Suárez J, Santín LJ, Rodríguez de Fonseca F, Castilla-Ortega E, García-Fernández MI, Serrano A. Plasma Concentrations of Lysophosphatidic Acid and Autotaxin in Abstinent Patients with Alcohol Use Disorder and Comorbid Liver Disease. *Biomedicines*. 2021 Sep 13;9(9):1207. doi: 10.3390/biomedicines9091207. PMID: 34572393; PMCID: PMC8469650.
4. Serrano-Castro PJ, Garzón-Maldonado FJ, Casado-Naranjo I, Ollero-Ortiz A, Mínguez-Castellanos A, Iglesias-Espinosa M, Baena-Palomino P, Sánchez-Sanchez V, Sánchez-Pérez RM, Rubi-Callejon J, Estévez-María JC, Galeano-Bilbao B, Romero-Imbroda J, Sobrino B, Arrabal-Gomez C, Oliver-Martos B, Muñoz-Becerra L, Requena N, González Álvarez de Sotomayor MDM, Estivill-Torrus G, Suarez J, Ciano-Petersen NL, Pons-Pons G, Reyes-Bueno JA, Cabezudo-Garcia P, Aguilar-Castillo MJ, De la Cruz Cosme C, Duque-Holguera M, Cuartero-Rodriguez E, Vilches-Carrillo RM, Carrera-Muñoz I, Carnero-Pardo C, Ramirez-Garcia T, Oropesa JM, Dominguez-Mayoral A, Pelaez-Viñas N, Valiente L, de Fonseca FR. The cognitive and psychiatric subacute impairment in severe Covid-19. *Sci Rep*. 2022 Mar 3;12(1):3563. doi: 10.1038/s41598-022-07559-9. PMID: 35241761; PMCID: PMC8894467.

5. Galván ST, Flores-López M, Romero-Sanchiz P, Requena-Ocaña N, Porras-Perales O, Nogueira-Arjona R, Mayoral F, Araos P, Serrano A, Muga R, Pavón FJ, García-Marchena N, de Fonseca FR. Plasma concentrations of granulocyte colony-stimulating factor (G-CSF) in patients with substance use disorders and comorbid major depressive disorder. *Sci Rep*. 2021 Jul 1;11(1):13629. doi: 10.1038/s41598-021-93075-1. PMID: 34211033; PMCID: PMC8249412.
6. García-Marchena N, Maza-Quiroga R, Serrano A, Barrios V, Requena-Ocaña N, Suárez J, Chowen JA, Argente J, Rubio G, Torrens M, López-Gallardo M, Marco EM, Castilla-Ortega E, Santín LJ, Rodríguez de Fonseca F, Pavón FJ, Araos P. Abstinent patients with alcohol use disorders show an altered plasma cytokine profile: Identification of both interleukin 6 and interleukin 17A as potential biomarkers of consumption and comorbid liver and pancreatic diseases. *J Psychopharmacol*. 2020 Nov;34(11):1250-1260. doi: 10.1177/0269881120928176. Epub 2020 Jun 13. PMID: 32536325.
7. Vidal R, García-Marchena N, O'Shea E, Requena-Ocaña N, Flores-López M, Araos P, Serrano A, Suárez J, Rubio G, Rodríguez de Fonseca F, Colado MI, Pavón FJ. Plasma tryptophan and kynurenine pathway metabolites in abstinent patients with alcohol use disorder and high prevalence of psychiatric comorbidity. Prog Neuropsychopharmacol *Biol Psychiatry*. 2020 Aug 30;102:109958. doi: 10.1016/j.pnpbp.2020.109958. Epub 2020 Apr 29. PMID: 32360814.
8. García-Marchena N, Pizarro N, Pavón FJ, Martínez-Huélamo M, Flores-López M, Requena-Ocaña N, Araos P, Silva-Peña D, Suárez J, Santín LJ, de la Torre R, Rodríguez de Fonseca F, Serrano A. Potential association of plasma lysophosphatidic acid (LPA) species with cognitive impairment in abstinent alcohol use disorders outpatients. *Sci Rep*. 2020 Oct 13;10(1):17163. doi: 10.1038/s41598-020-74155-0. PMID: 33051508; PMCID: PMC7555527.

9. Flores-López, M., Porras-Perales, O., Requena-Ocaña, N., García-Marchena, N., Araos, P., Serrano, A., ... & Pavón, F. J. (2021). Inflammatory factors and depression in substance use disorder. In *The Neuroscience of Depression* (pp. 149-160). Academic Press.