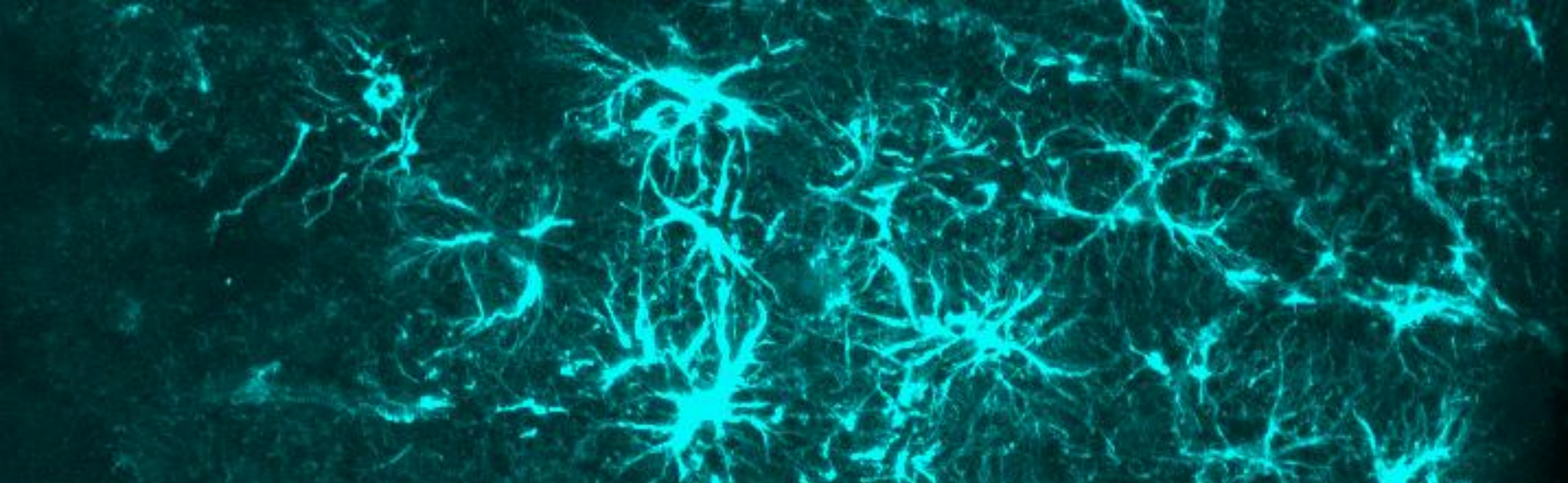




The Aryl Hydrocarbon Receptor (AhR) as a biomarker and therapeutic target in post-stroke cognitive impairment

Carmen Nieto Vaquero, PhD Student

Grupo Fisiopatología Neurovascular (CNIC), Dra. María A. Moro

Unidad de Investigación Neurovascular (UCM, i+12), Dr. Ignacio Lizasoain y

Dra. Maribel Cuartero

Proyectos colaborativos para investigadores emergentes



Meet AhR: The dioxin receptor

The Aryl Hydrocarbon Receptor (AhR)

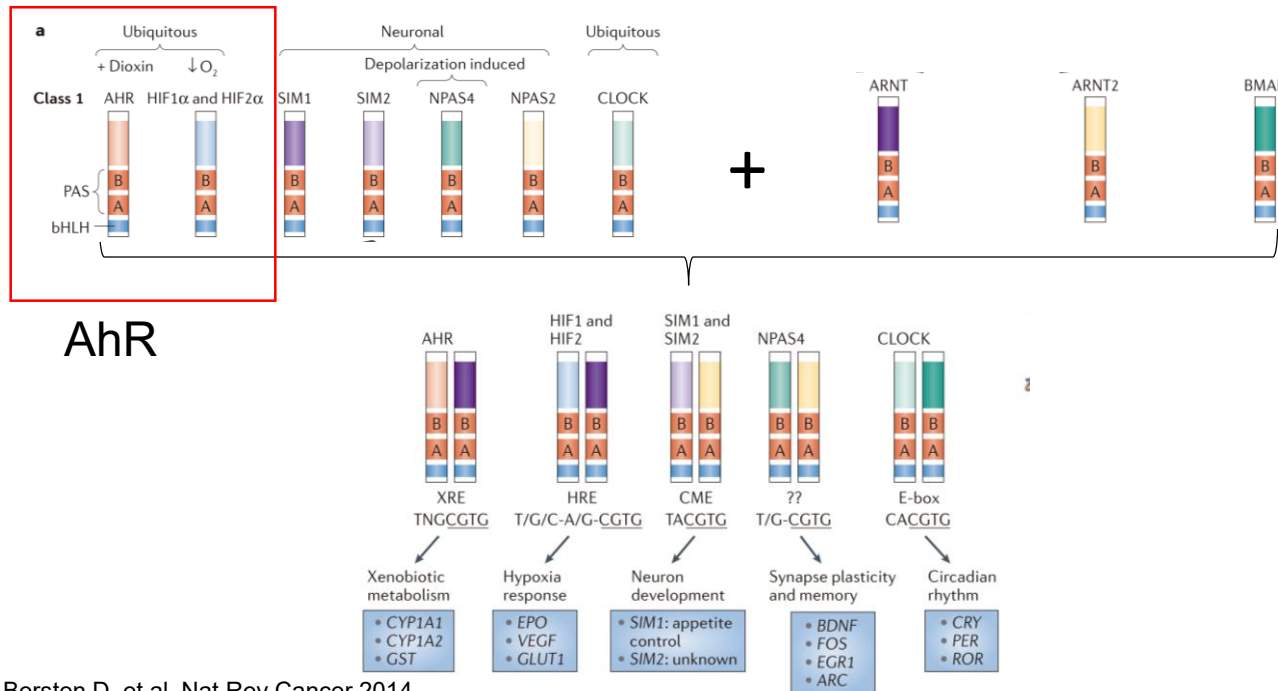
Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor

(2,3,7,8-tetrachlorodibenzo-*p*-dioxin/aryl hydrocarbon receptor nuclear translocator/Sim/Per/helix-loop-helix)

KRISTINE M. BURBACH*, ALAN POLAND†, AND CHRISTOPHER A. BRADFIELD*‡

*Department of Pharmacology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611; and †McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, WI 53706

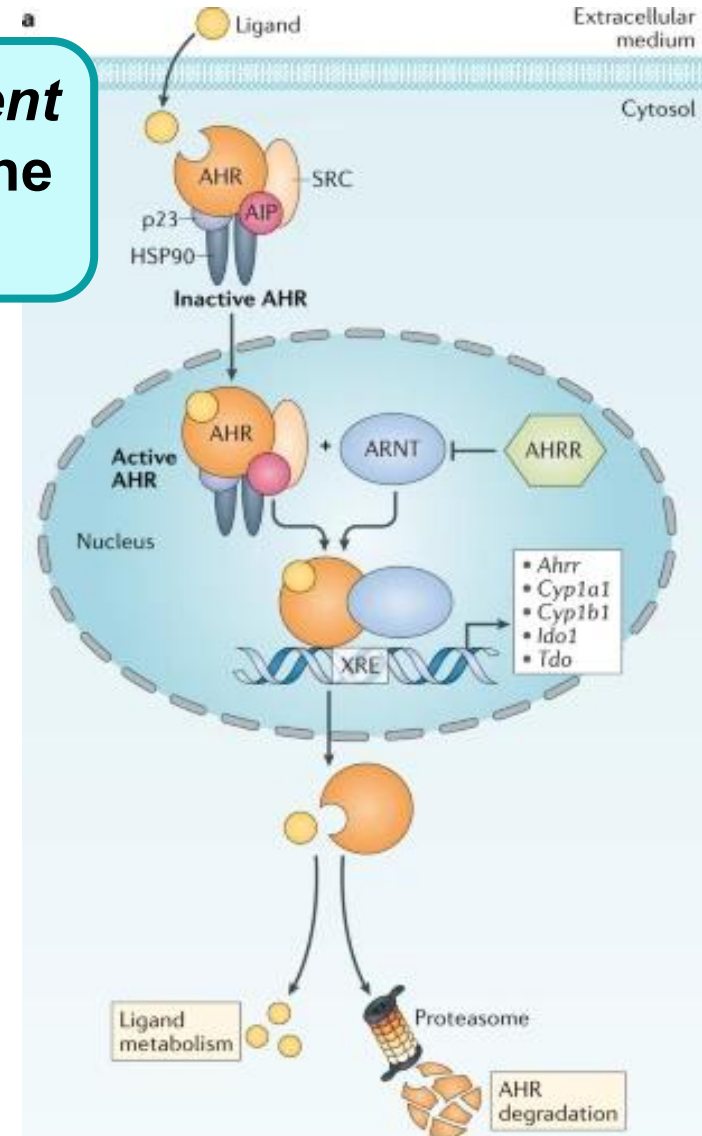
The basic HLH (helix-loop-helix) –PER–ARNT–SIM (bHLH–PAS) family



Bersten D. et al. Nat Rev Cancer 2014

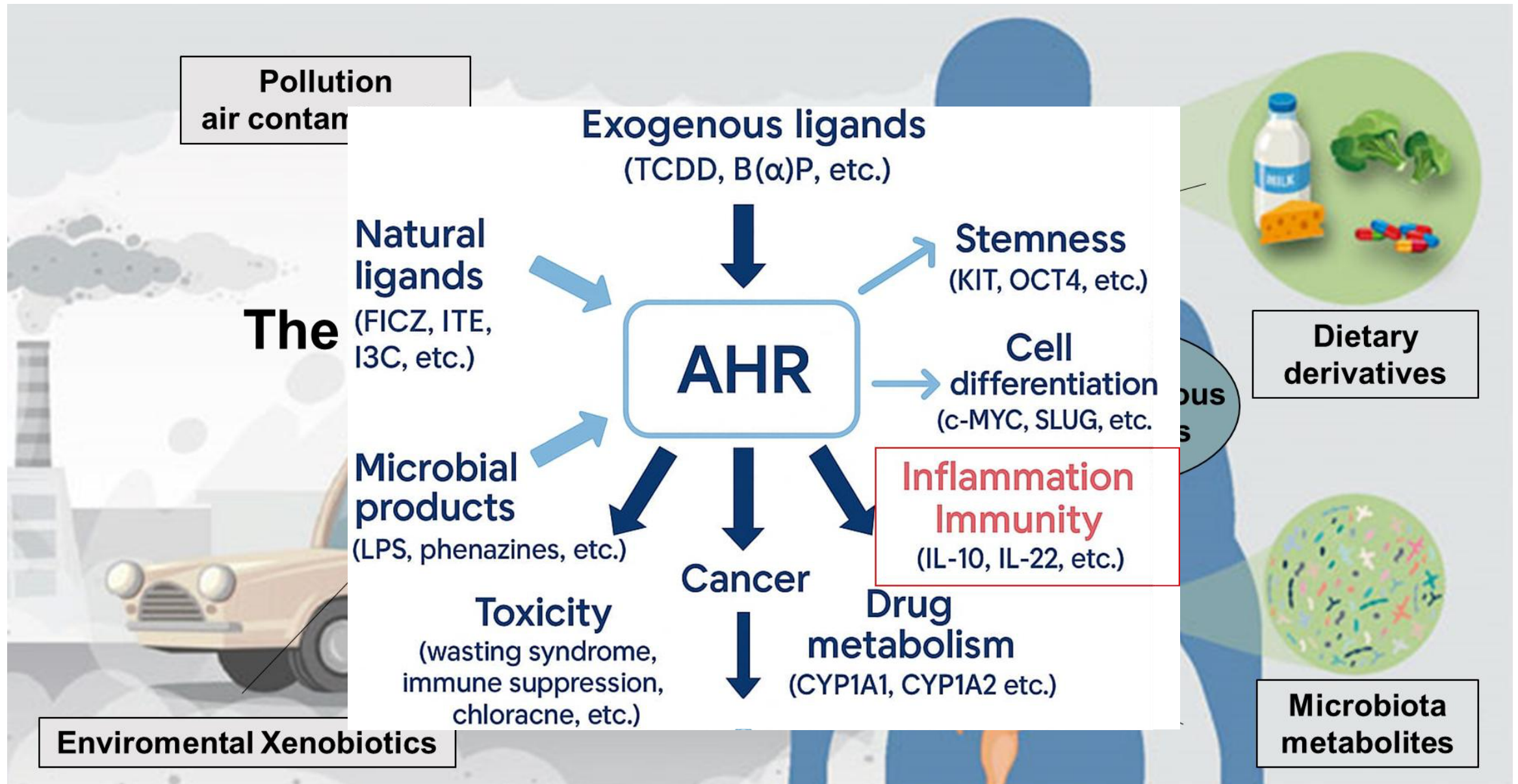
The AhR pathway and signaling

XRE-dependent control of gene expression



Rothhammer and Quintana., Nat Rev Immunol 2019

A Sensor of the External and Internal Microenvironment



Post-stroke biomarkers

We propose that AhR or its ligands may serve as potential biomarkers

STROKE



Oligodendrocytes
MMP9

Neurons
NSE
NR2A/2B aABs
NR2
MMP9
Glycogen phosphorylase isoenzyme BB
Glutamate

Microglia
MMP9
MCP-1

All cells
PARK7/DJ-1
NDKA
Caspase-3
GST-π

Endothelium
PAI-1
TAF1
VCAM
s-RAGE

Neurodegenerative and
y biomarkers
ent light (Nfl)
protein (CRP)
IL)

OUTCOME

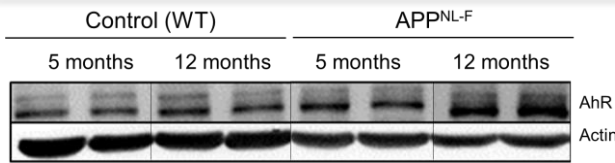
Andone S. et al. Int J Mol Sci 2021

Immune response
in the acute phase

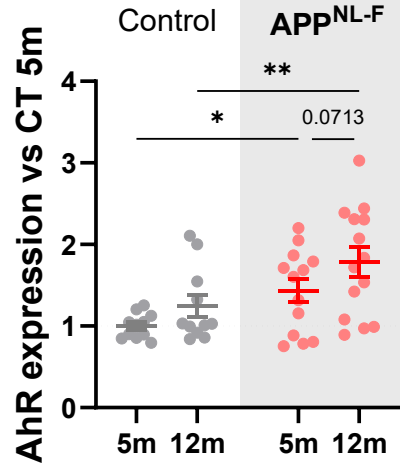
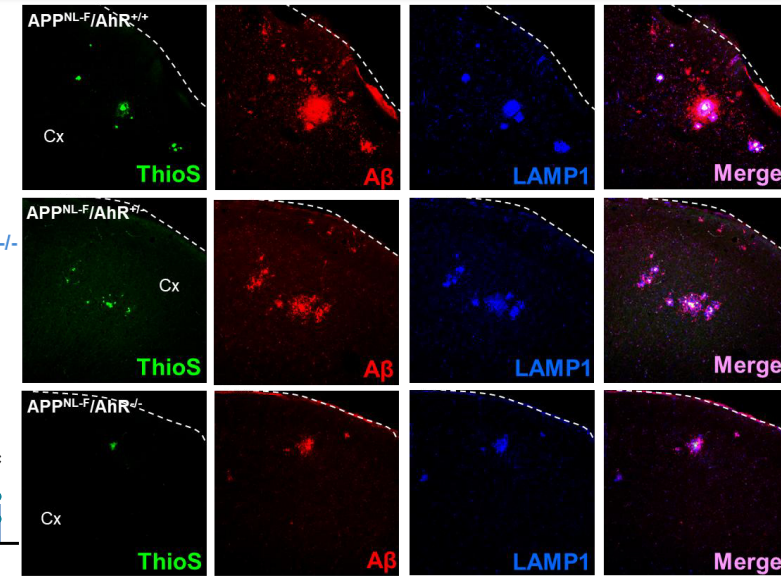
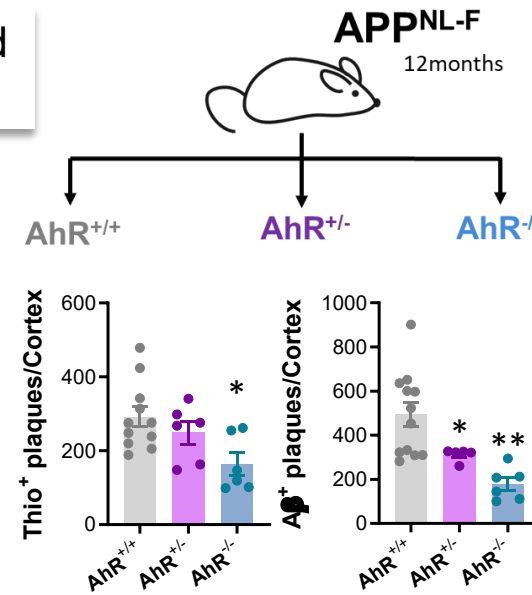
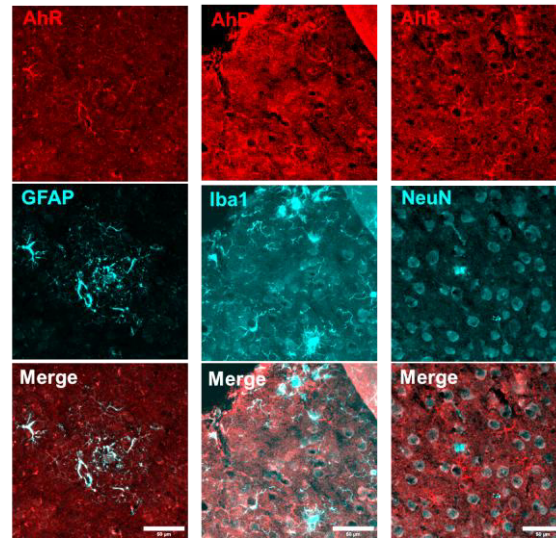
Chronic **maladaptive**
responses

Cognitive
impairment

Microglial AhR drives Alzheimer's disease progression

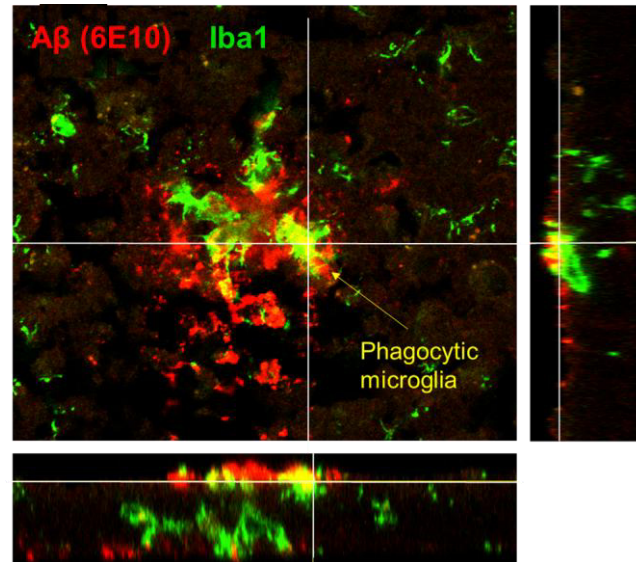
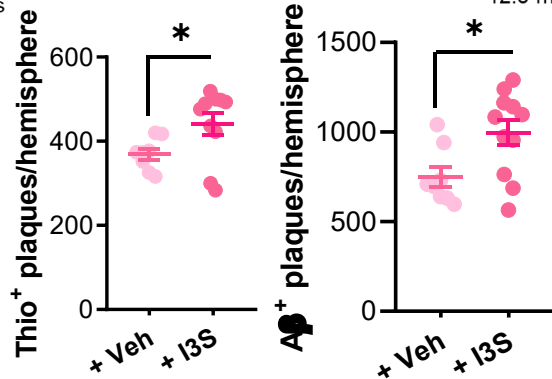
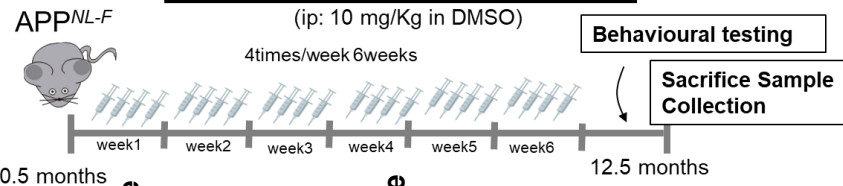


AhR expression is associated to glial cells

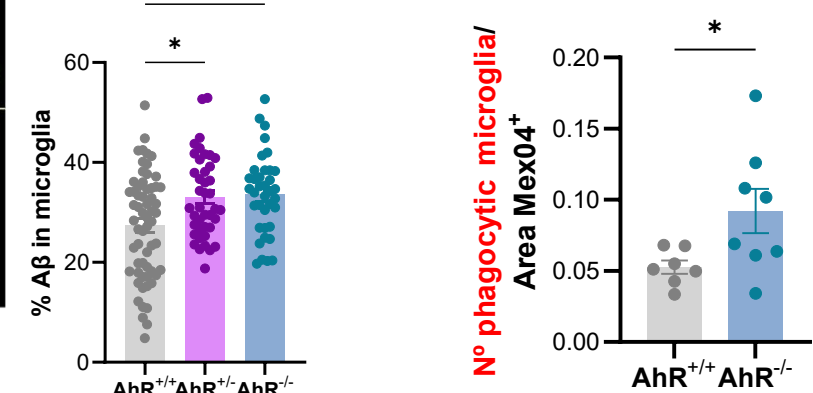
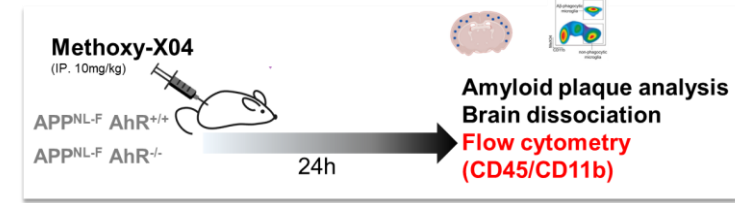


Chronic I3S administration

(ip: 10 mg/Kg in DMSO)

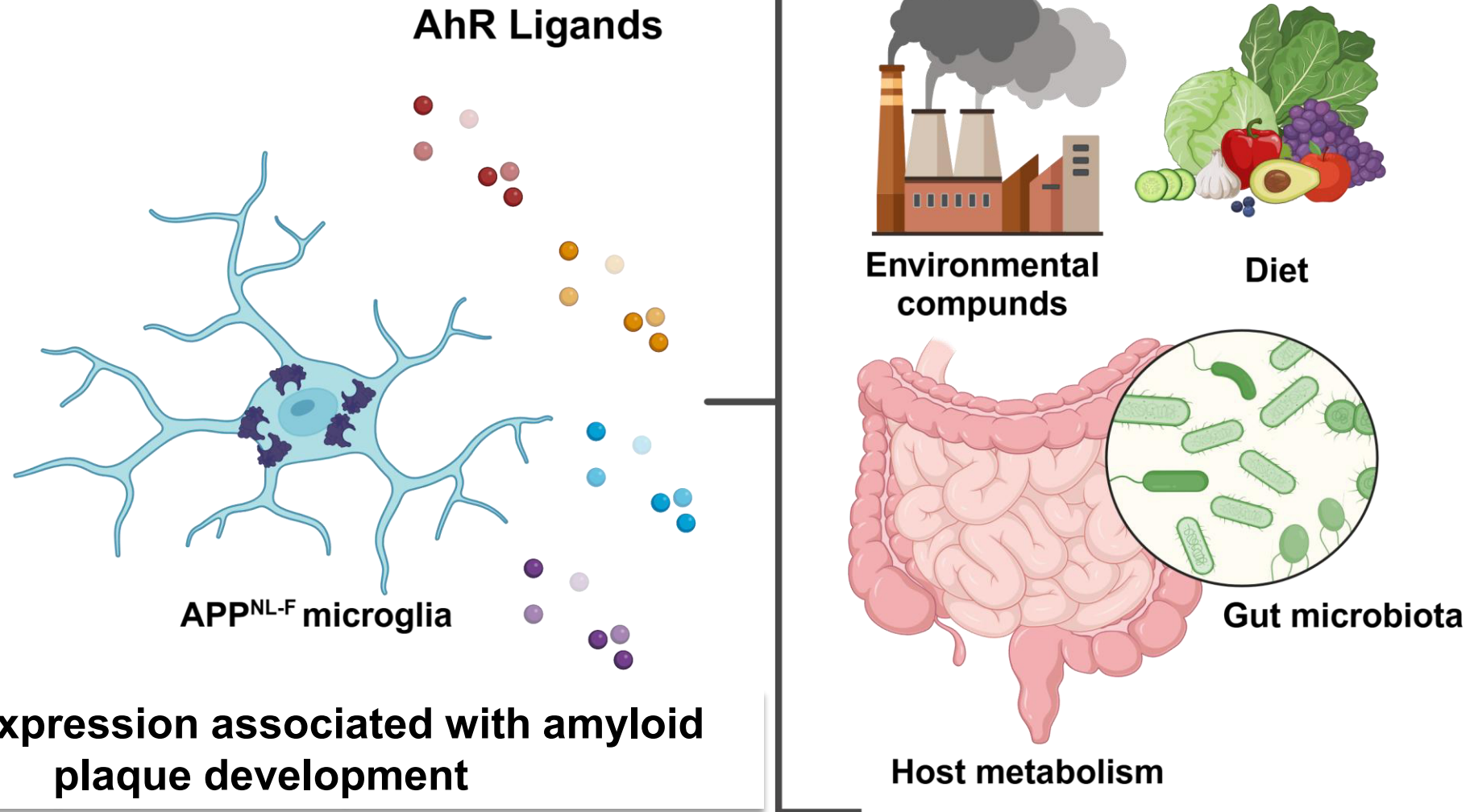


In vivo assay of phagocytosis



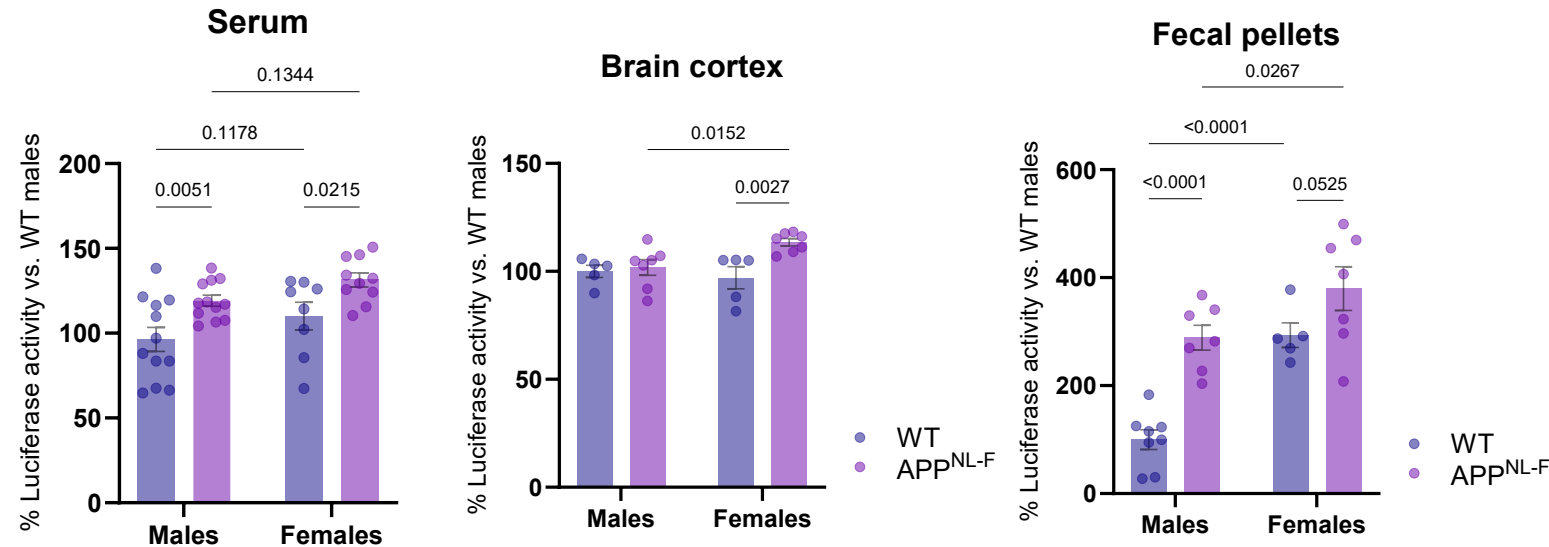
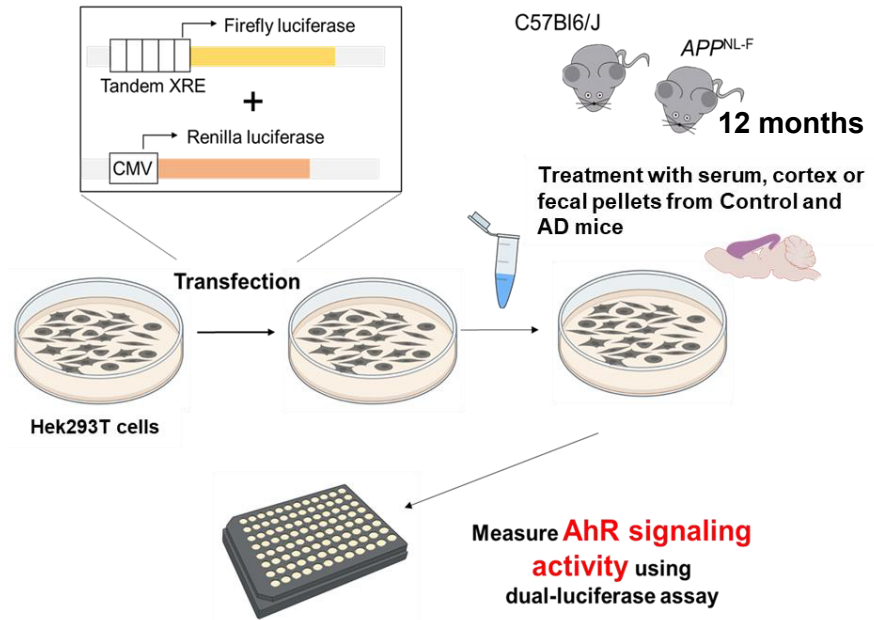
Nieto-Vaquero and cols. Unpublished data

How does AhR signaling modulate microglial function?



Are AhR ligands increased in Alzheimer's disease??

AhR ligands levels change with AD



AhR Activity and metabolomics

Colaboration with Dr. Alberto Villarejo Galende

Imaging Study

- CT and MRI
- Dat-Scan
- PET-amyloid
- DaTScan
- Amyloid-PET
- PET-FDG
- EEG

Cognitive Study

- Clinical Dementia Rating
- Mini-Mental State Examination
- Verbal phonetic fluency test
- Trail making test
- Inverse Digits of the WAIS-III
- Free and Cued Selective Reminding Test
- Boston naming test
- Hamilton Depression Scale

Blood and LCR Samples

- Glucemia
- Cholesterol
- HDL and LDL
- triglycerids
- HbA1c
- hsCRP
- Hemoglobin
- Leukocytes
- Fibrinogen
- ACT-prothrombin
- TTPA
- Beta amiloyd
- AB42 and AB40
- t-tau and p-tau
- Cociente pTau/AB42
- YKL-40
- sTREM2
- NFL
- GFAP
- NSE
- BDNF

Inclusion criteria

Alzheimer patients: Age over 40 years.
b) Alzheimer's disease according to NIA-AA criteria, both in the deterioration phase mild cognitive impairment and dementia

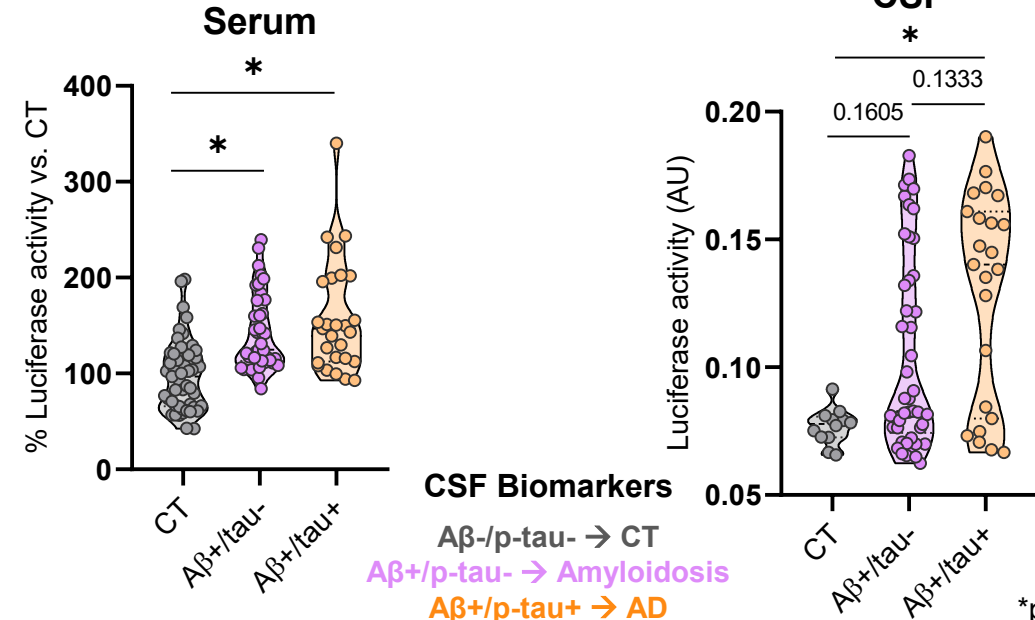
Controls: Age over 40 years; Absence of cognitive complaints; Plasma Tau181 levels below the cut-off point

Exclusion criteria

Previous neurodevelopmental delay. Major psychiatric illness: major depression (present), schizophrenia (present or past), bipolar disorder (present or past), obsessive-compulsive disorder (present or past), according to the DSM-V. Substance abuse (present) (DSM-V-TR). Presence of infectious, inflammatory, tumor or traumatic disease.

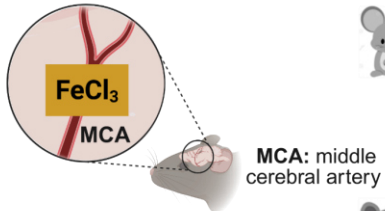
Recruitment at Neurology service of the **University Hospital October 12**

Ongoing

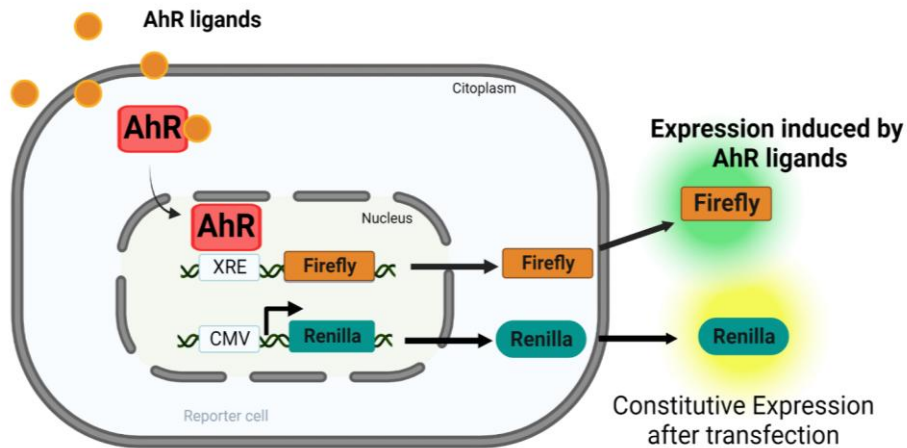
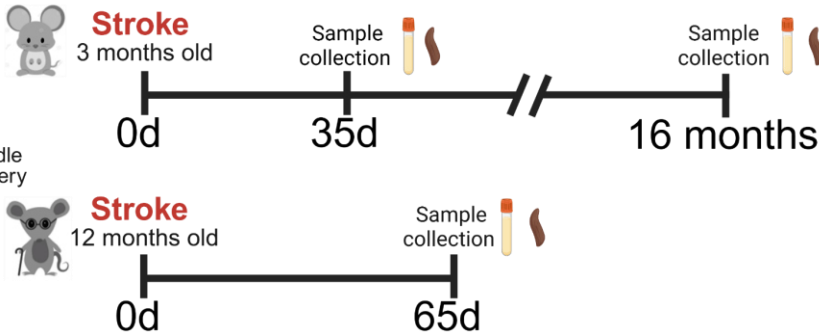


*p < 0.05 ; n = 5-35

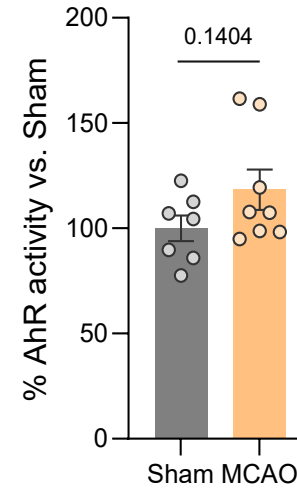
AhR ligands levels change in mice after stroke



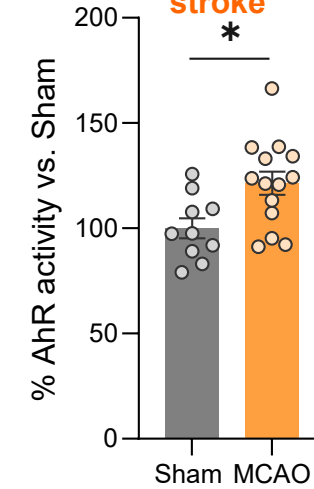
MCA: middle cerebral artery



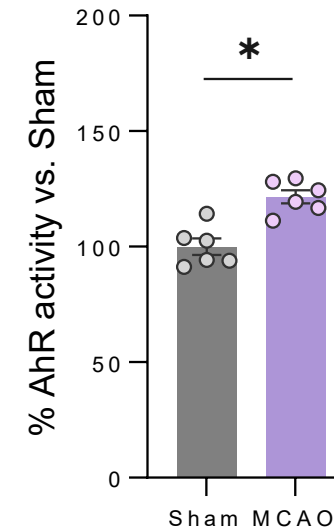
Young mice 35d after stroke



Young mice 16 months after stroke



Old mice 65d after stroke

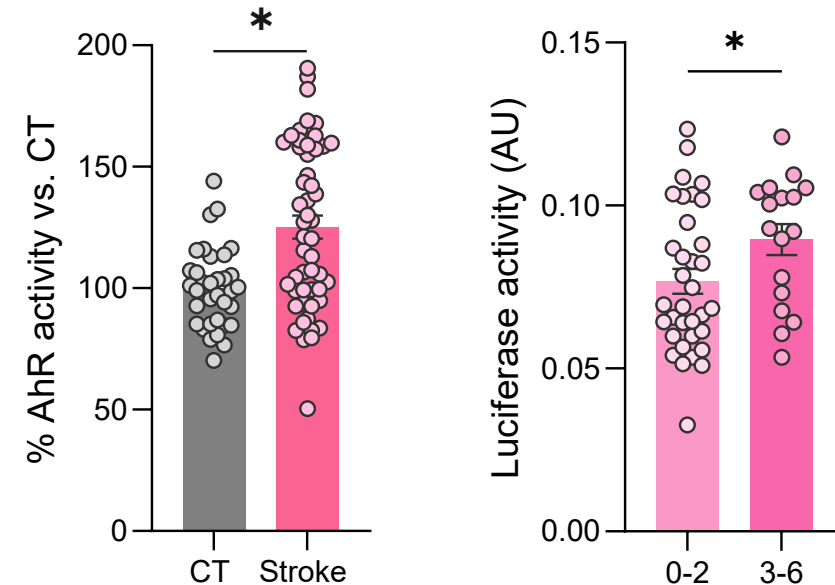
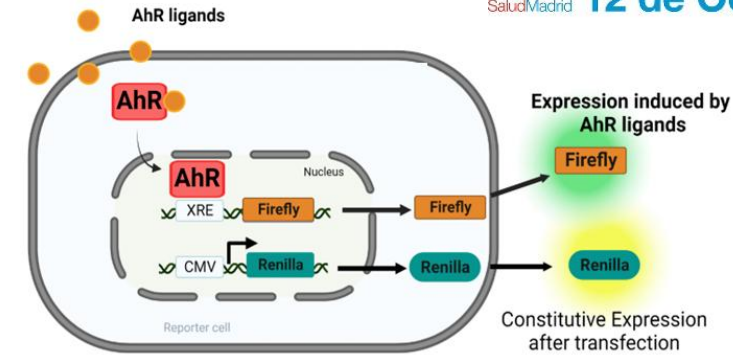


AhR ligands levels change in patients after stroke

STROKE DATABASE – 3 MONTHS AFTER STROKE PILOT STUDY

Variable	n = 55
Age – mean ± SD	74.71 ± 11.99
Female sex – n (%)	27 (49.09%)
Smoking – n (%)	13 (24.07%)
Alcohol – n (%)	6 (11.11%)
Hypertension – n (%)	37 (67.27%)
Dyslipidemia – n (%)	31 (56.36%)
Diabetes – n (%)	13 (23.64%)
Pre-stroke functional Independence (mRS 0-2) – n (%)	55 (100%)
Baseline NIHSS – mean ± SD	10.53 ± 7.54
NIHSS at 24h – mean ± SD	5.04 ± 4.27
tPA – n (%)	22 (39.62%)
Thrombectomy – n (%)	31 (55.56%)
Etiology (TOAST) – n (%)	
Atherothrombotic	21 (38.18%)
Cardioembolic	19 (34.55%)
Lacunar	1 (1.82%)
Cryptogenic	6 (10.91%)
Multiple causes	6 (10.91%)
Other cause	2 (3.64%)
Incomplete study	0 (0%)
3-month poor outcome (mRS 3-6) – n (%)	18 (32.73%)

Preliminary data



Modified Rankin Scale 3 months
after stroke

- Good outcome mRS 0-2
- Poor outcome mRS 3-6

Proyectos colaborativos para investigadores emergentes

Hypothesis

Aryl Hydrocarbon Receptor (AhR) ligands are altered in patients and animal models of ischemic stroke, and these alterations are associated with **long-term cognitive and functional outcomes**, as well as with the development of **post-stroke cognitive impairment**. Therefore, signaling through the AhR, and specifically the detection of its agonist activity, could be used as a **biomarker of clinical progression in ischemic stroke patients**.

Objectives

To clarify the **relationship** between **AhR ligands** and **post-stroke cognitive impairment** by analyzing these ligands in **animal models** and in **patient samples**.

1. To evaluate AhR ligands in an animal model of ischemic stroke.

2. To characterize the role of AhR in chronic ischemic stroke in mice.

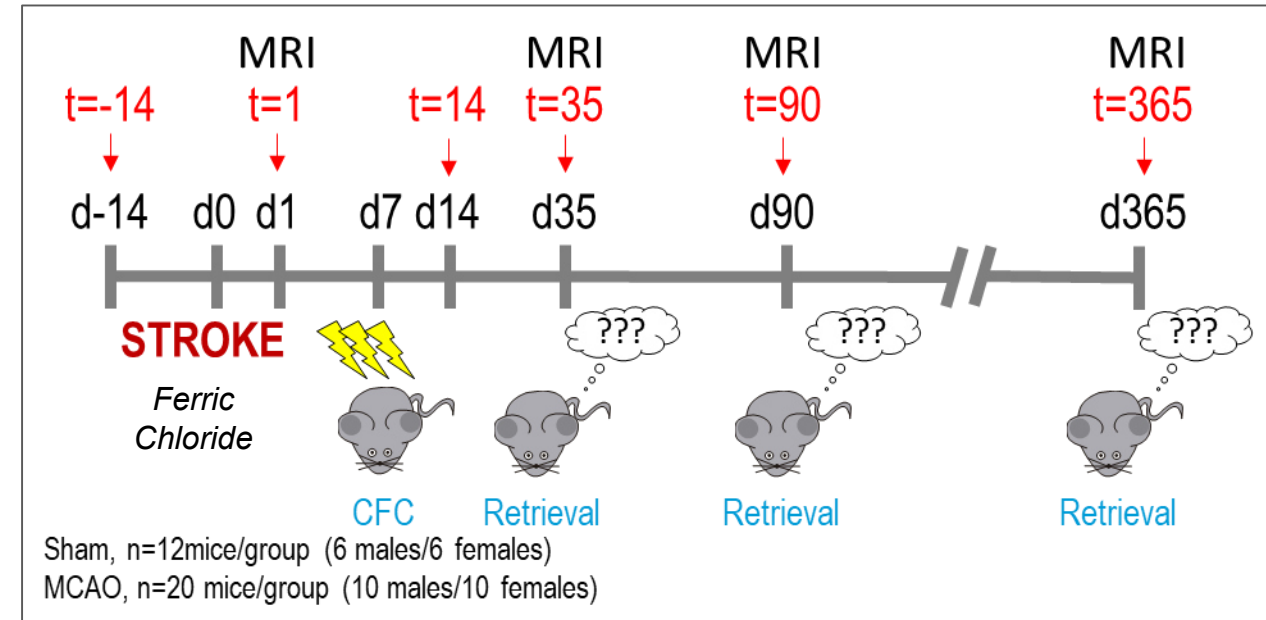
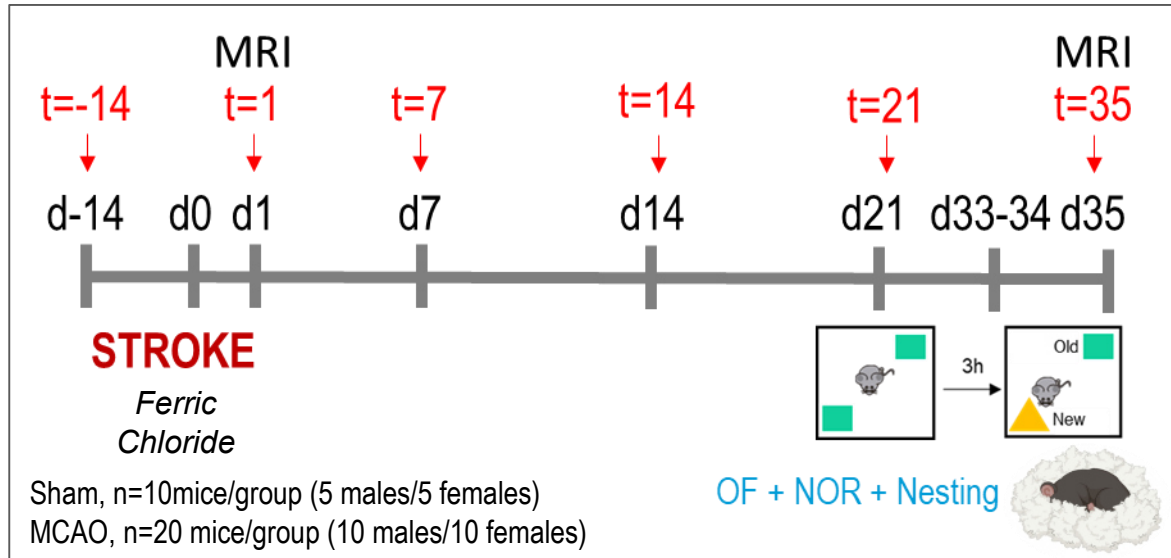
3. To determine AhR ligands in post-stroke patient samples.

Characterization of AhR ligands in preclinical models of stroke

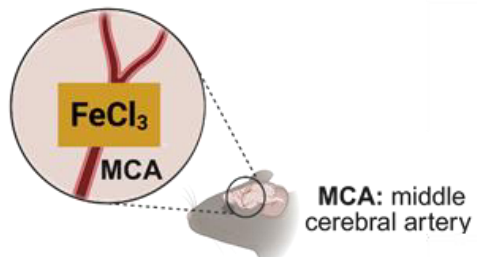


1. To evaluate AhR ligands in an animal model of ischemic stroke

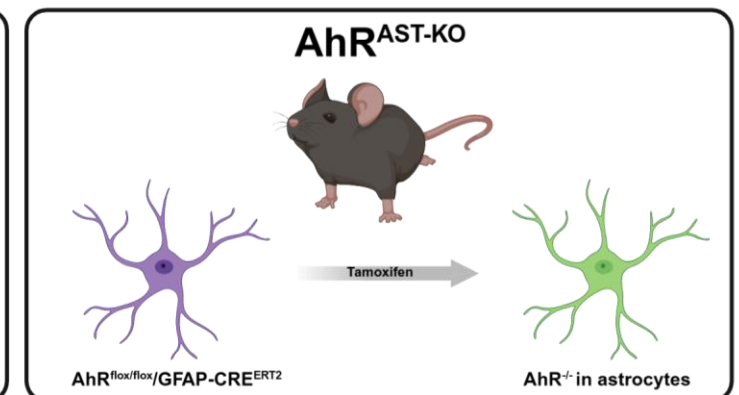
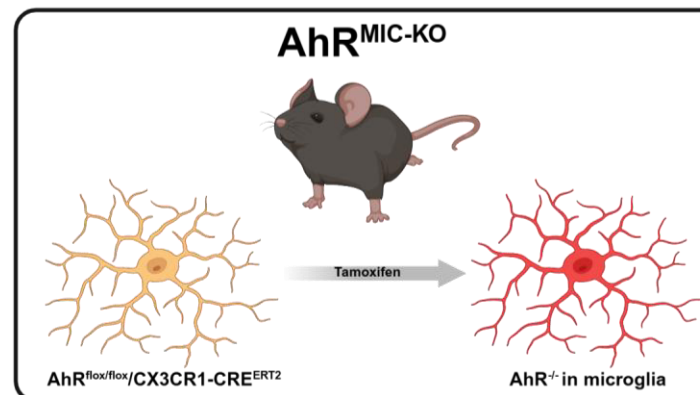
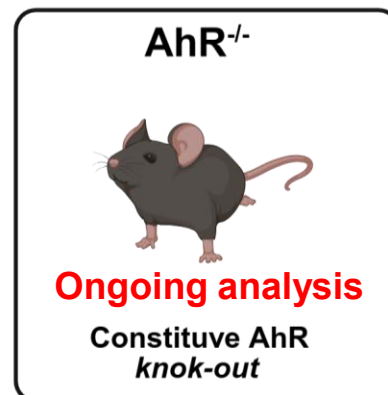
Ongoing analysis



2. To characterize the role of AhR in chronic ischemic stroke in mice



MCA: middle cerebral artery



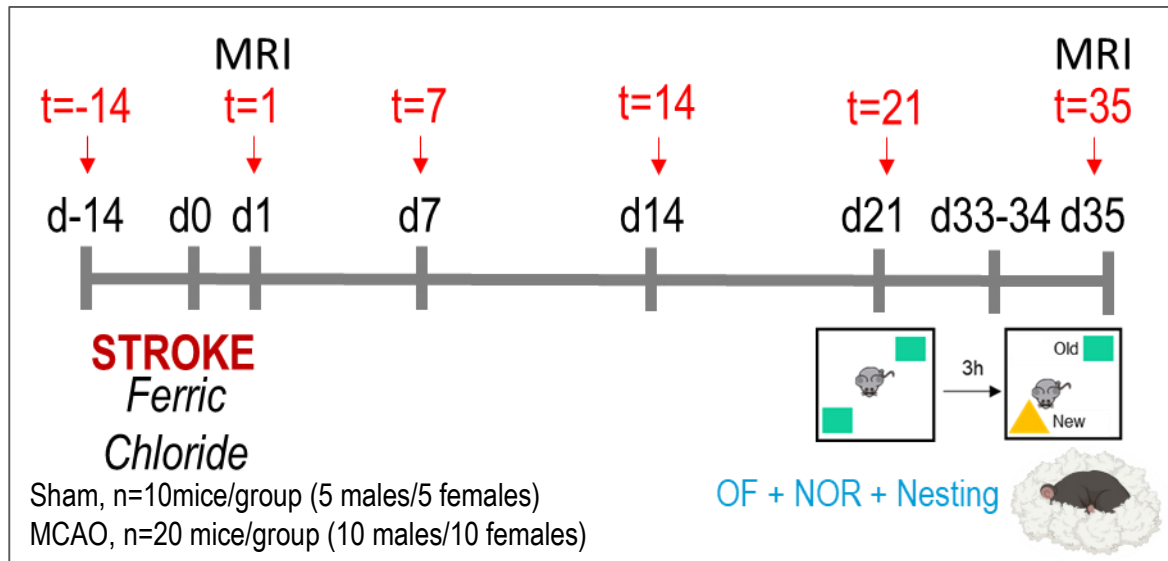
Characterization of AhR ligands in preclinical models of stroke

1. To evaluate AhR ligands in an animal model of ischemic stroke

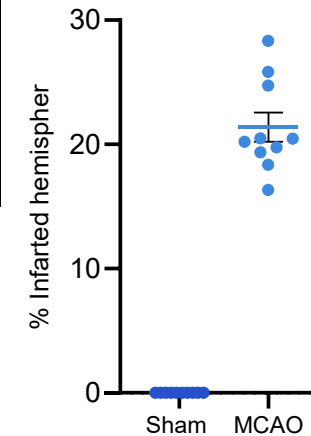
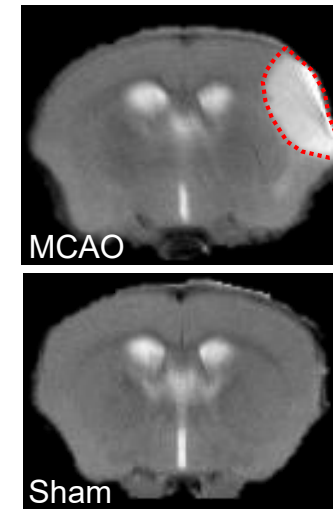


Carlos Parra (Surgeries), Virginia García (Behaviour test), Laura Martínez (Imaging)

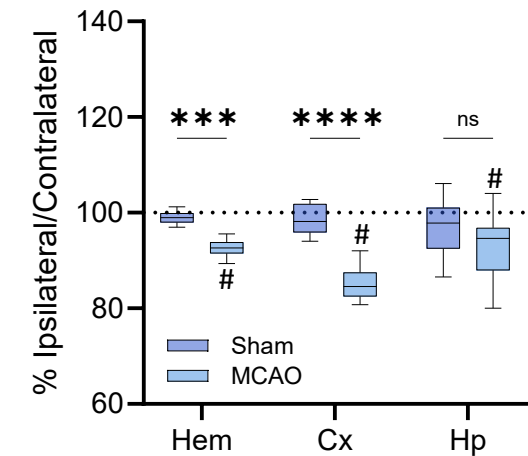
Ongoing analysis



MRI 24h



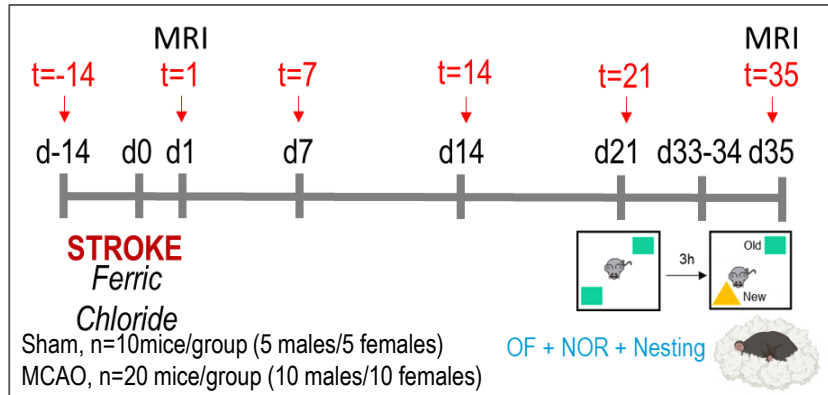
MRI 35d



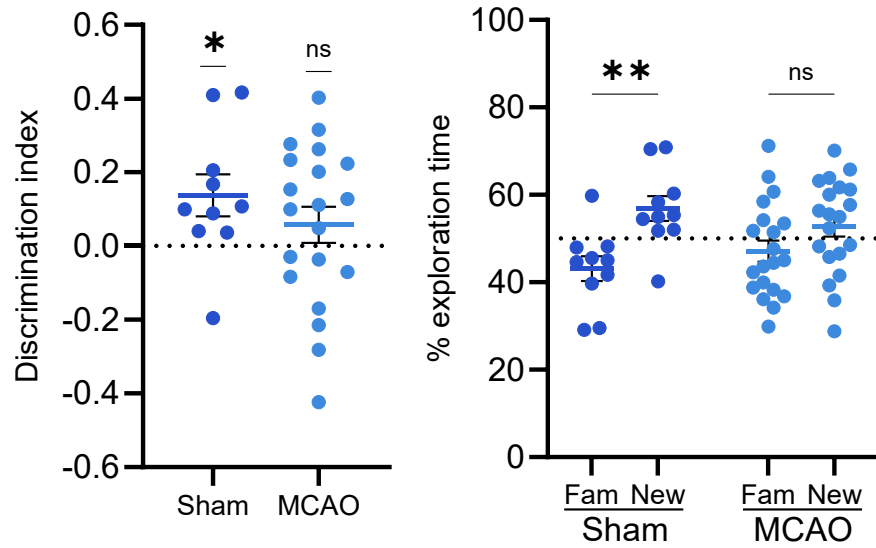
Characterization of AhR ligands in preclinical models of stroke

1. To evaluate AhR ligands in an animal model of ischemic stroke

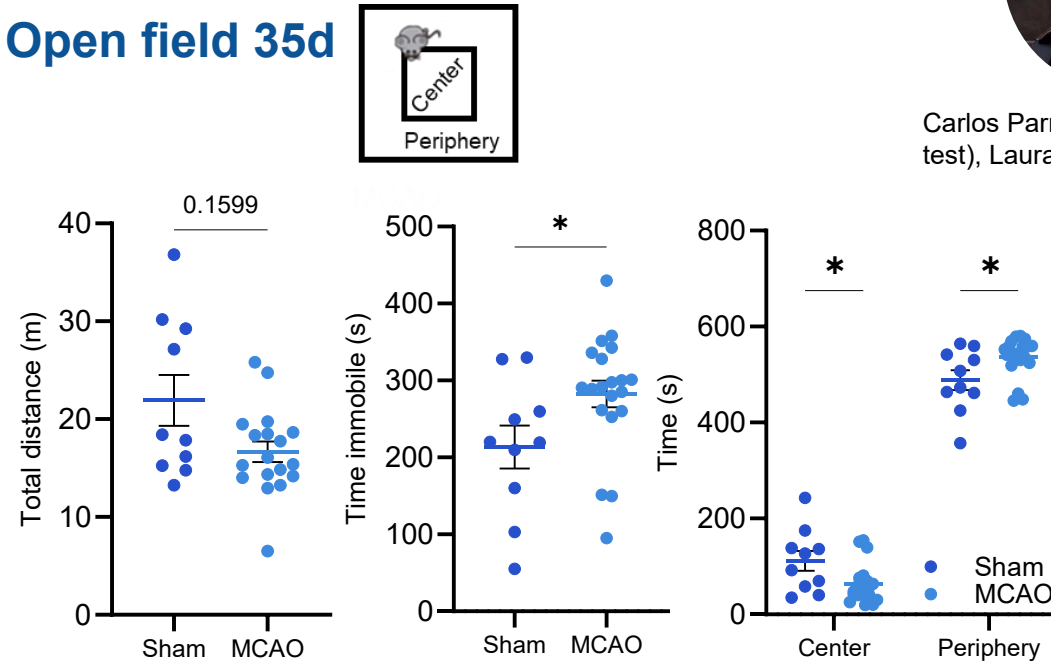
Ongoing analysis



Novel object recognition test 35d



Open field 35d

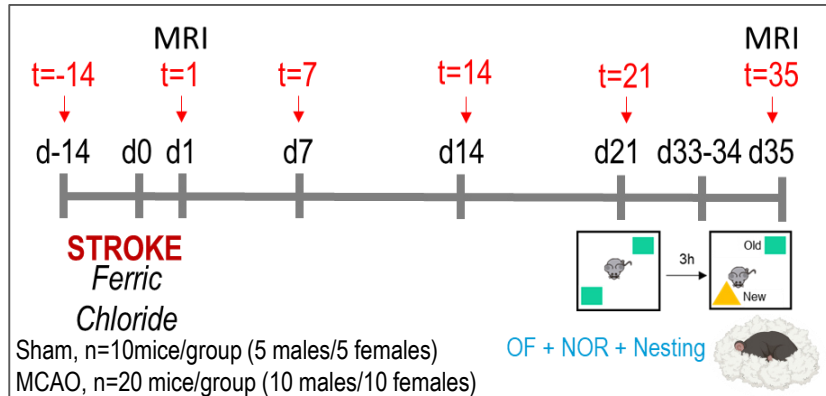


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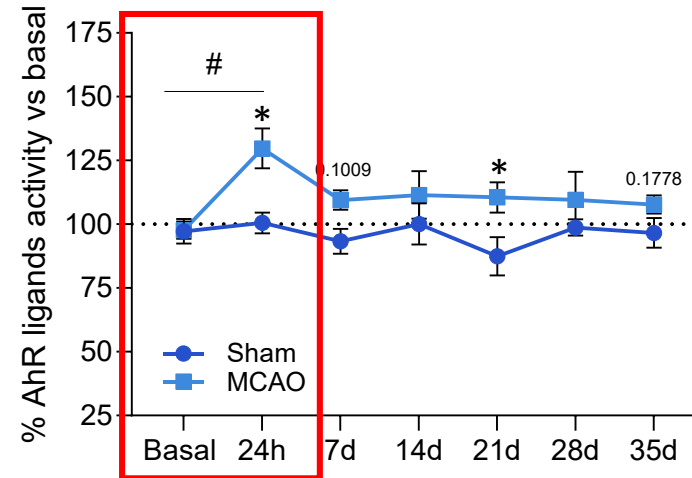
Characterization of AhR ligands in preclinical models of stroke

1. To evaluate AhR ligands in an animal model of ischemic stroke

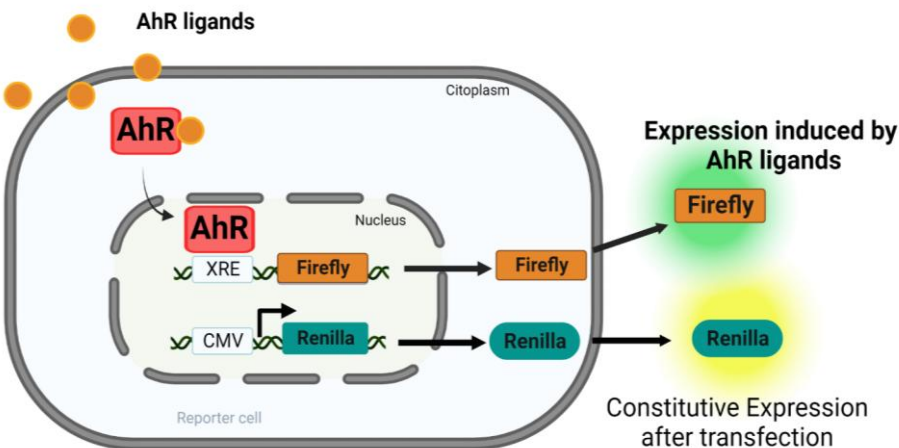
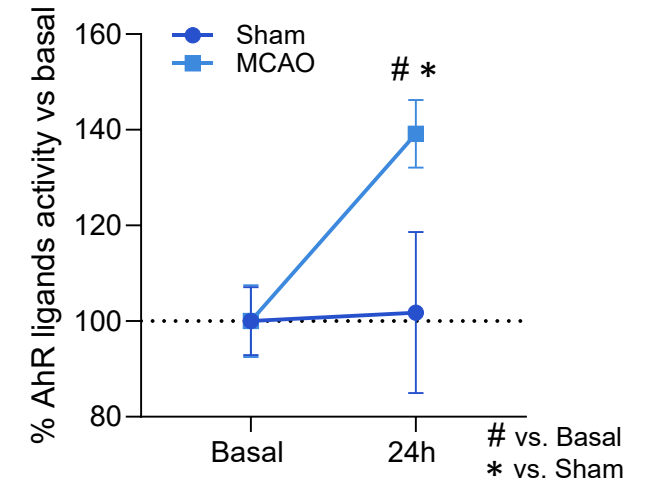
Ongoing analysis



AhR ligands in serum



AhR ligands in fecal pellets

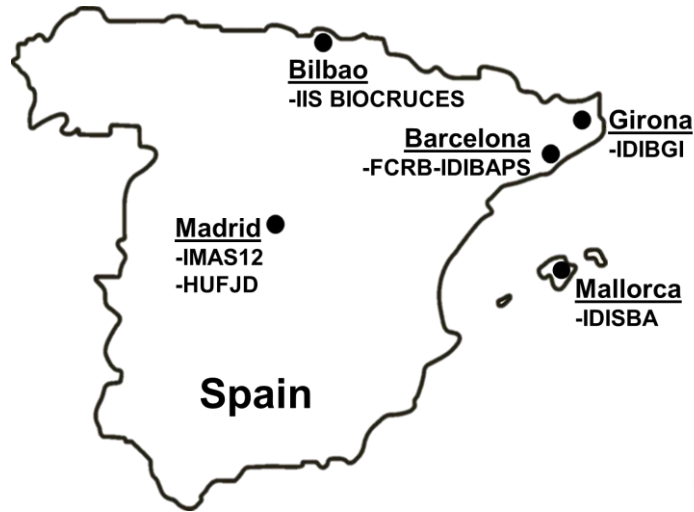


Ongoing:

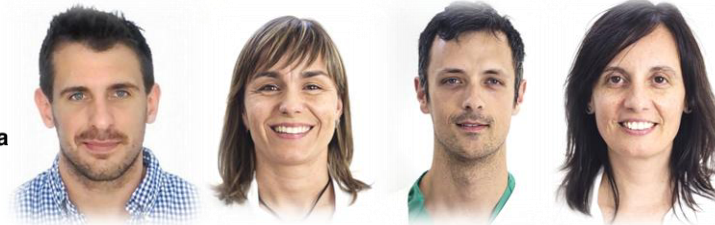
- AhR ligands activity in feces and brain
- Correlate AhR ligands-Cognitive impairment

Evaluation of circulating AhR ligands in stroke patients

3. To determine AhR ligands in post-stroke patient samples



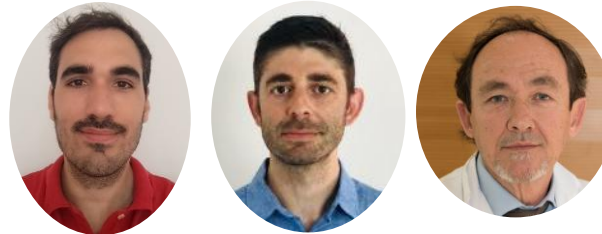
IDIBGI
Institut d'Investigació Biomèdica de Girona
Dr. Josep Trueta



Juan Álvarez-Cienfuegos Rodríguez, Carme Gubern Mérida y Mikel Terceño Izaga (Yolanda Silva Blas)

CEIM favorable, MTA en preparación UCM-Biobanco

IDIBAPS
Institut D'Investigacions Biomèdiques August Pi i Sunyer
FUNDACIÓ CLÍNIC
BARCELONA



Antonio Doncel-Moriano Cubero y Salvatore Rudilosso (Ángel Chamorro Sánchez)

**CEIM favorable,
MTA gestionándose UCM-IDIBAPS**

IdISBa
Institut d'Investigació Sanitària Illes Balears

Aryan Naghedi Jafari
(Cristòfol Vives Bauzà)

i+12
Institut de Investigación
Hospital 12 de Octubre

cnic



Maribel Cuartero (Ignacio Lizasoain y María Ángeles Moro)

CEIM favorable – 24/03/2026

Hospital Universitario **Fundación Jiménez Díaz**
Grupo **quirónsalud**



Marta Oses-Lara (Araceli García Torres)

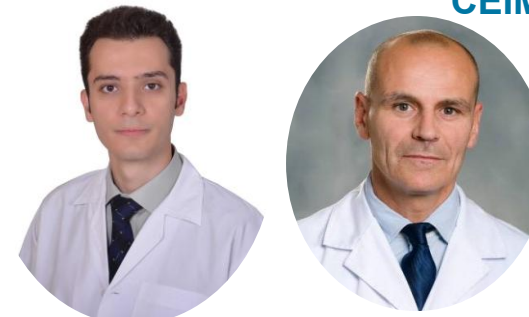
CEIM en preparación

b+ocruces bizkaia
osasun ikerketa institutua
instituto de investigación sanitaria



Jon Martín Prieto (María del Mar Freijo Guerrero)

CEIM en preparación

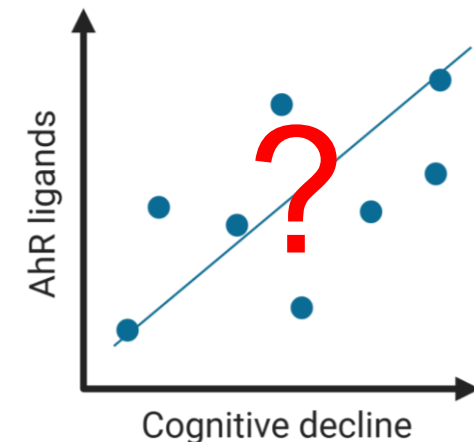
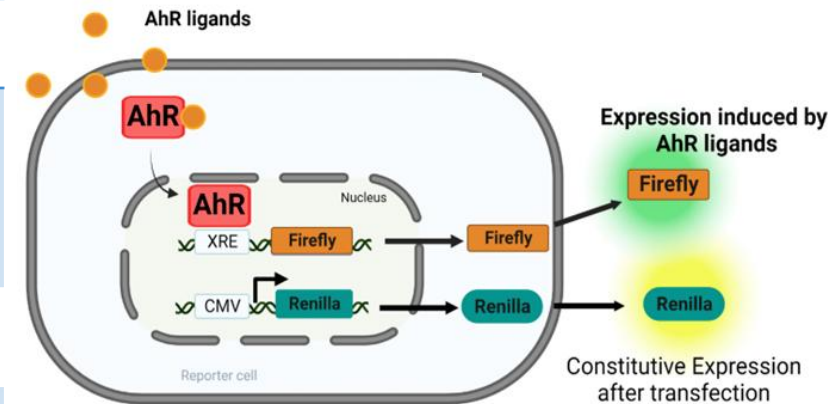


Muestras en Madrid para analizar

Evaluation of circulating AhR ligands in stroke patients

3. To determine AhR ligands in post-stroke patient samples

Centro	Nº de muestras (% mujeres; edad media)	Etiología	Puntos temporales de las muestras	Escalas cognitivas y funcionales
IMAS12	N =55 (49.09%; 74.71 años)	Aterotrombótico 38.18%, Cardioembólico 34.55%, Lacunar 1.82%, Criptogénico 10.91, Coexistencia de causas 10.91% y Causa inhabitual 3.64%	Suero a los 90 días	NIHSS basales, 24h y 3 meses; ERm previo y 3 meses
IIS BIOCRUCES	Por determinar	Por determinar	Suero basal, 24h y 3 meses	NIHSS basal, 24h, alta y 3 meses
FCRB	N = 62 (17%; 72 años)	Lacunares 50% y pequeñas lesiones isquémicas no lacunares 50%	Suero y microbiota basales y/o 24h Suero 1 año	MOCA, DSST, TMT A&B, 24-72h y 1 año. ERm basal, 3 meses y 1 año
HU FJD	N = 31 (19,35%; 73,9 años)	Aterotrombótico 38.71%, Cardioembólico 32,26%, Lacunar 9,68%, e indeterminado 19,35%	Suero y plasma, basal, 24h, 48h y 90 días	ERm y NIHSS, basal, 24h, 48h y 90 días
IDIBGI	N = 49 (49%; 74,5 años)	Aterotrombótico 22,4%, cardioembólico 69,4%, y Criptogénico 8,2%	Suero basal, 24h y 3 meses	NIHSS basal, 24h, alta, y 3 meses. ERm previo y 3 meses



IMAS12 → 54 pacientes con muestras de suero basales y 24h (5 excluidos hemorrágicos) → 8 citados para revisión a 3 meses (abril-agosto)

IDISBA → 51 pacientes con muestras de suero basales/24h y revisión a los 3 meses

CNIC

María Ángeles Moro

Andrea Rubio

Sandra Vázquez

Carmen Nieto

Javier de Castro

Carlos Parra

Aurora Semerano

Eneko Merino

Francisco José Cantero

Tania Jareño

Virginia García

Laura Martínez

UCM - i+12

Ignacio Lizasoain

Maribel Cuartero

Alicia García-Culebras

Jesús Pradillo

Ana Moraga

Macarena Hernández-Jiménez

Manuel Navarro

Blanca Díaz

Cristina Granados

Fernando Ostos

Nuria Alfageme

Lidia García

URJC

Carolina Peña-Martínez

RICORs

Juan Álvarez-Cienfuegos Rodríguez,

Carme Gubern Mérida y Mikel

Terceño Izaga (Yolanda Silva Blas)

Antonio Doncel-Moriano Cubero y

Salvatore Rudilosso (Ángel

Chamorro Sánchez)

Jon Martín Prieto (María del Mar

Freijo Guerrero)

Marta Osés Lara (Araceli García

Torres)

Aryan Naghedi Jafari (Cristòfol

Vives Bauzà)



Collaborations

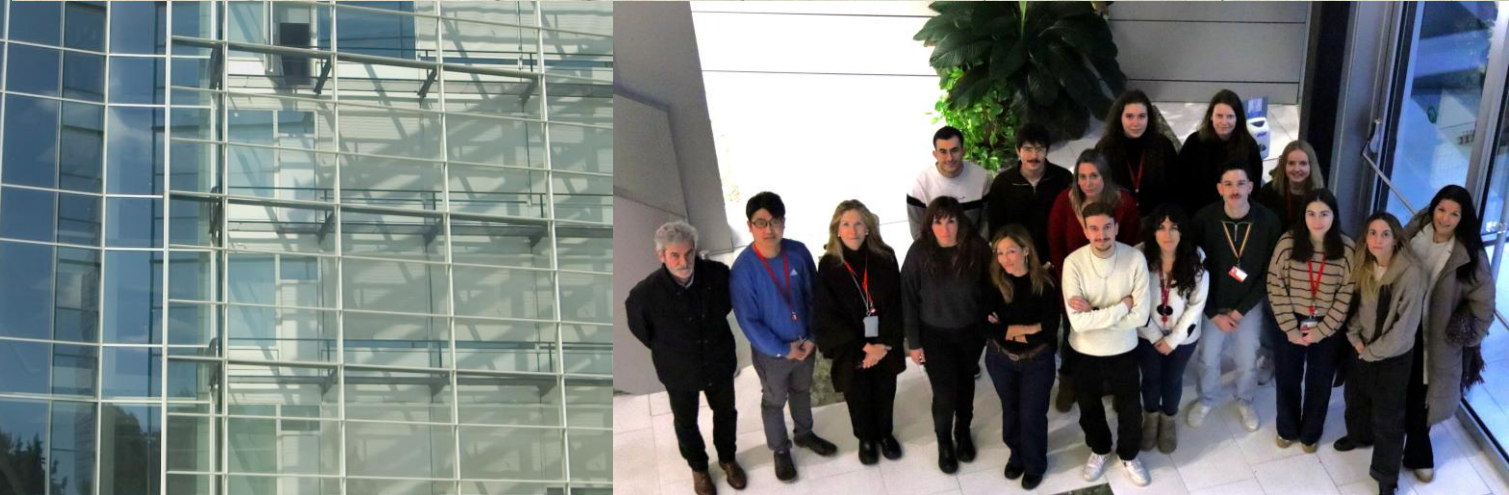
Alberto-Villarejo (i+12)

Pedro Fernández-Salguero (UNEX)

Carlos G. Dotti (CBMSO)

Takaomi C. Saido (RIKEN)

Takashi Saito (Nagoya City Univ)



Grant PID2022-140616OB-I00

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Comunidad de Madrid
Grant PIPF-2022/
SAL-GL-26119

