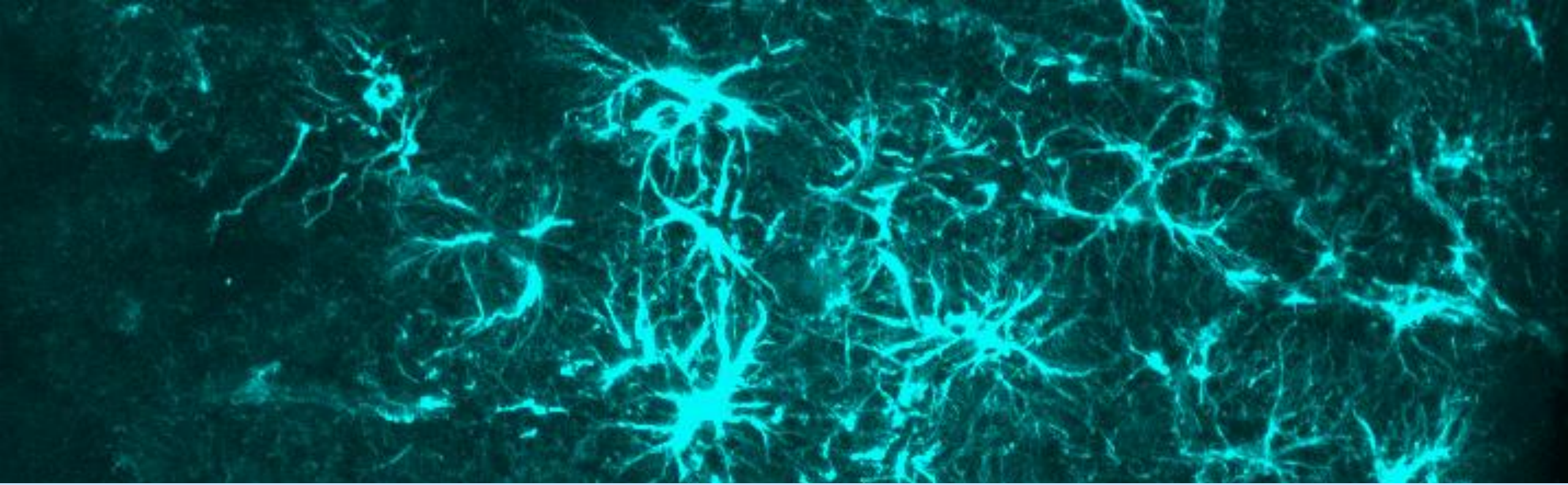


The Aryl Hydrocarbon Receptor (AhR) as a biomarker and therapeutic target in post-stroke cognitive impairment

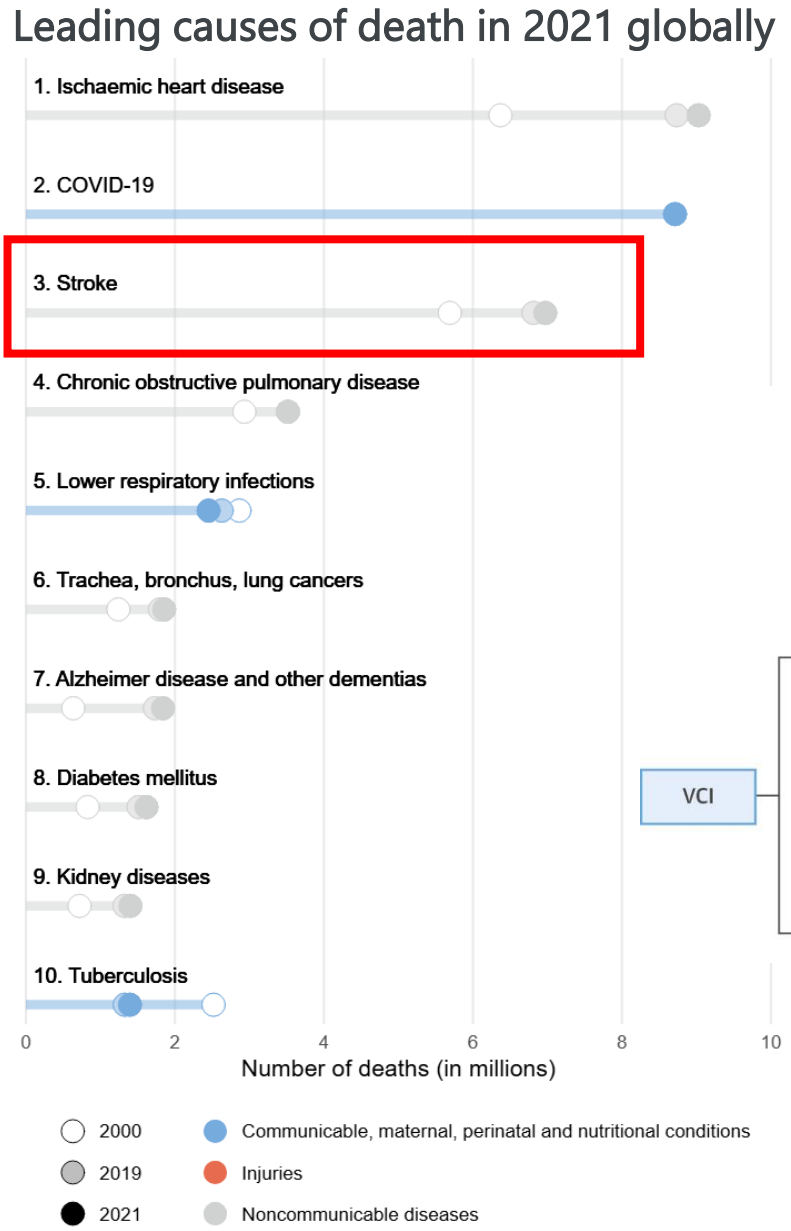
Carmen Nieto Vaquero, PhD Student

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

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

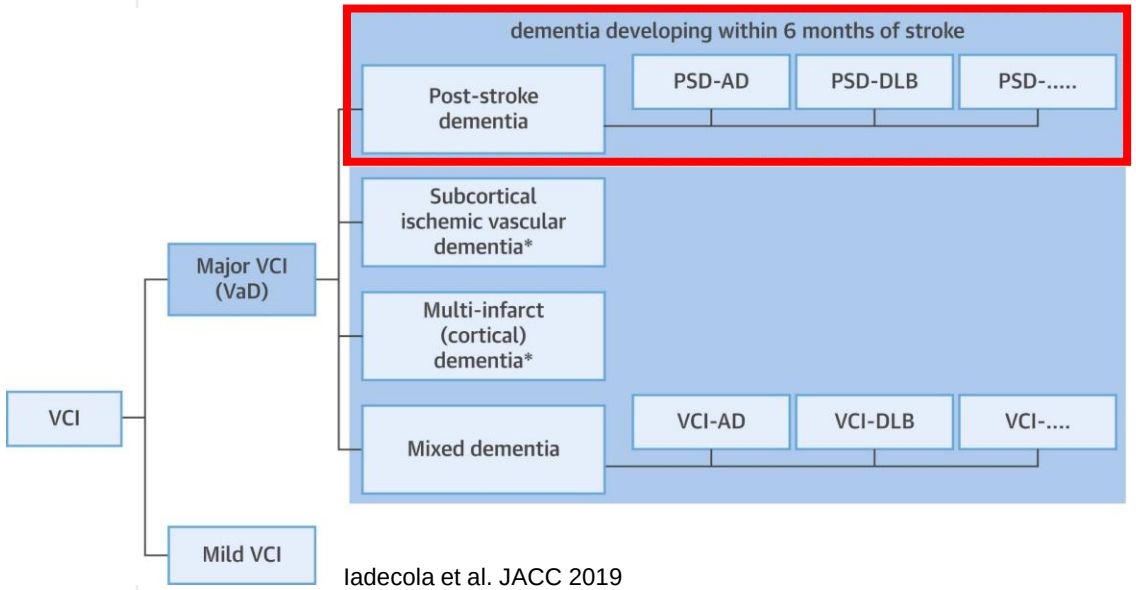
Proyectos colaborativos para investigadores emergentes

Stroke impact

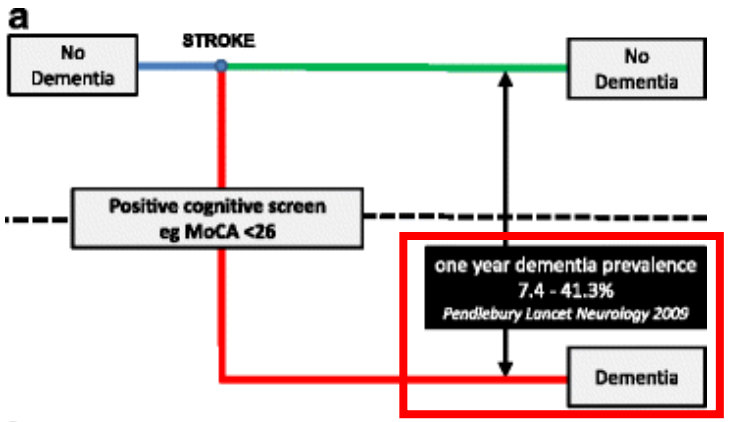


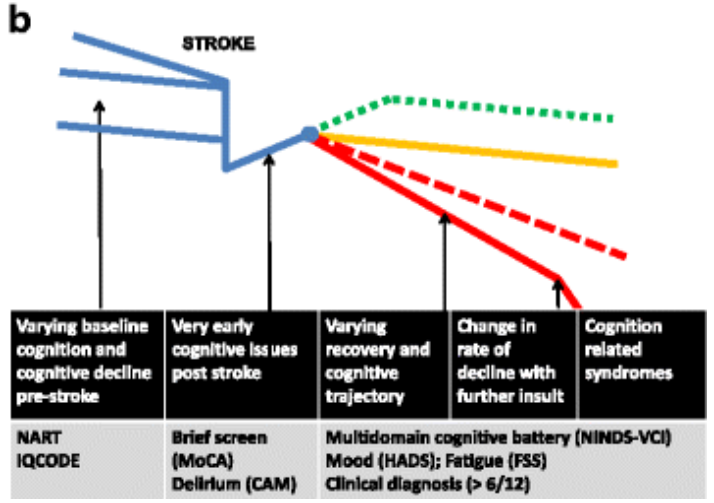
"Stroke remains the second-leading cause of death and the third-leading cause of death and disability combined in the world"

World Stroke Organization (WSO): Global Stroke Fact Sheet 2022



Iadecola et al. JACC 2019





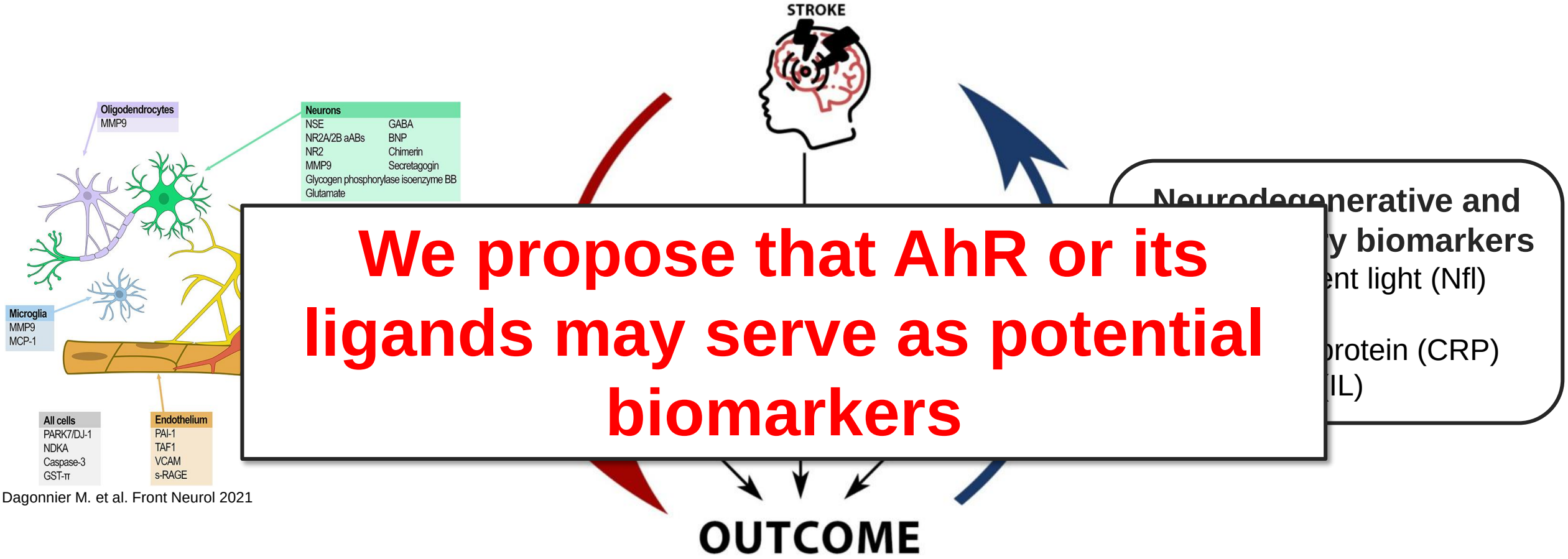
Mijajlović, M. D. et al. BMC Med 2017

Post-stroke cognitive impairment

3-6 months; 30%

WHO Global Health Estimates, 2021 Rost N. et al. Circ Res 2022; Jokinen H. et al. Eur J Neurol 2015; Iadecola C. Neuron 2013

Post-stroke biomarkers



Dagonnier M. et al. Front Neurol 2021

Andone S. et al. Int J Mol Sci 2021

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

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

Immune response in the acute phase → Chronic **maladaptive responses** → **Cognitive impairment**

Tiedt S. et al. Neurology 2018; Brodtmann A.et al. Front Neurol 2021; Peng Y. and cols. Neurorehabil Neural Repair 2021

Meet AhR: The dioxin receptor

The Aryl Hydrocarbon Receptor (AhR)

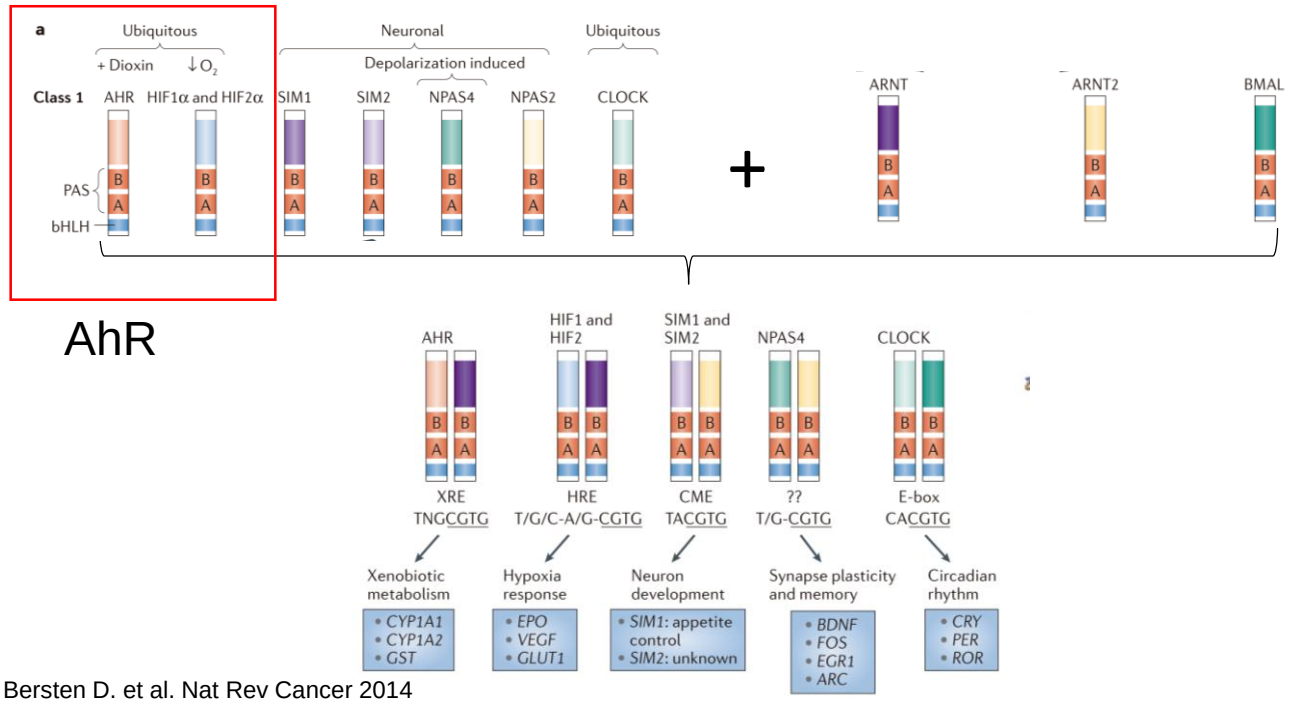
Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor

(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

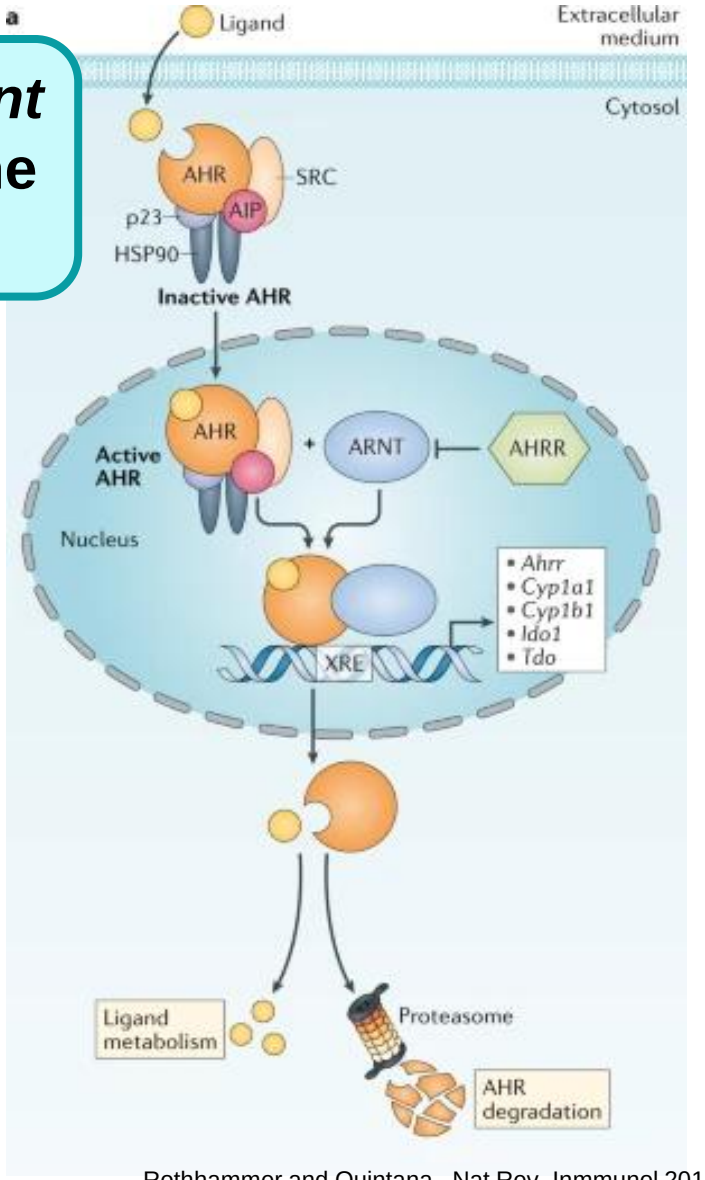
The basic HLH (helix–loop–helix) –PER–ARNT–SIM (bHLH–PAS) family



Bersten D. et al. Nat Rev Cancer 2014

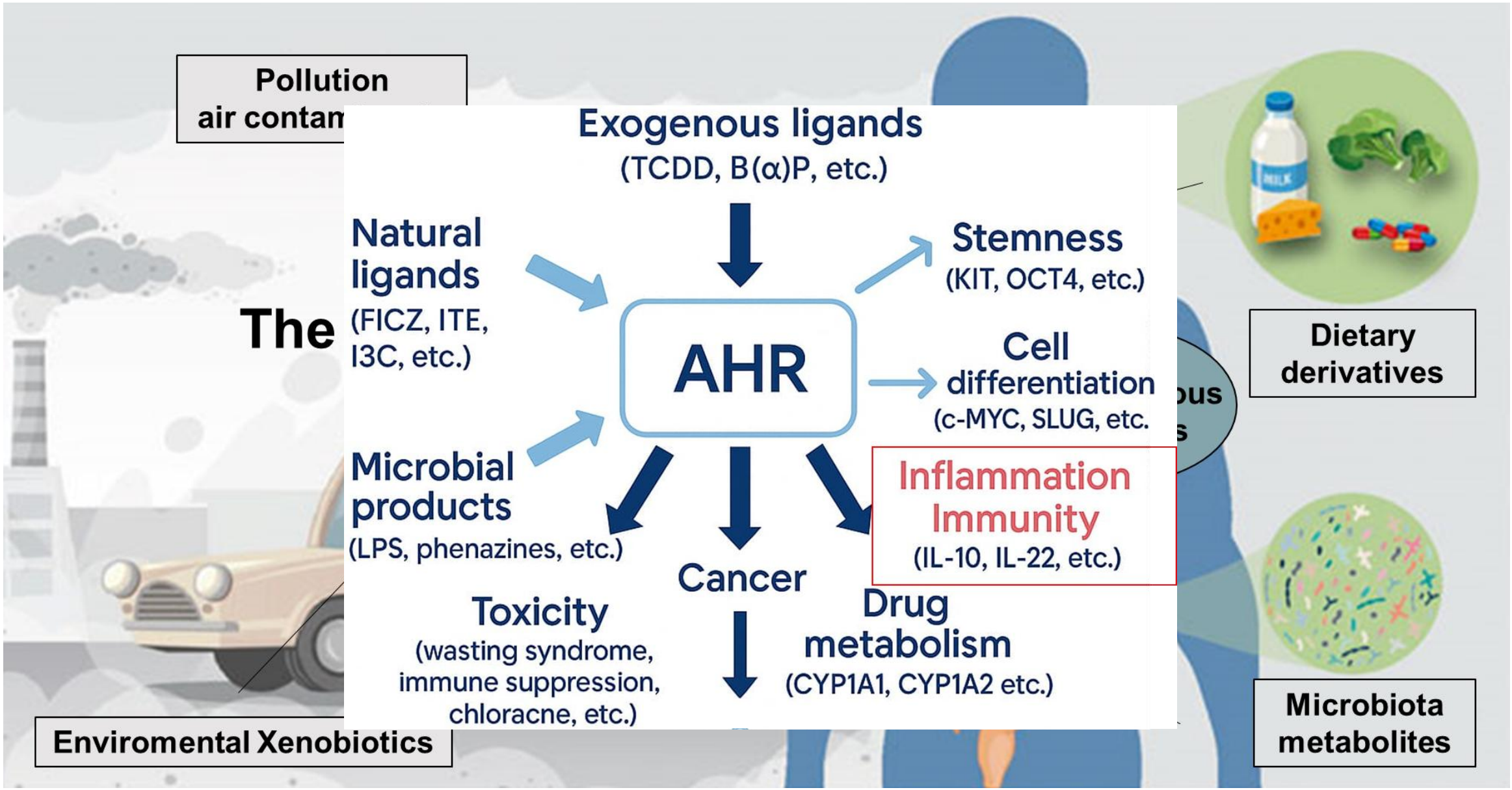
The AhR pathway and signaling

XRE-dependent **control of gene expression**



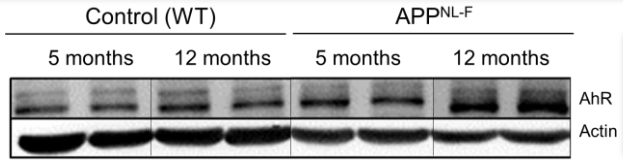
Rothhammer and Quintana., Nat Rev Inmmunol 2019

A Sensor of the External and Internal Microenvironment

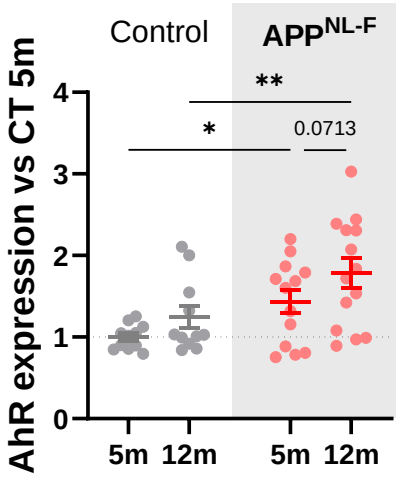


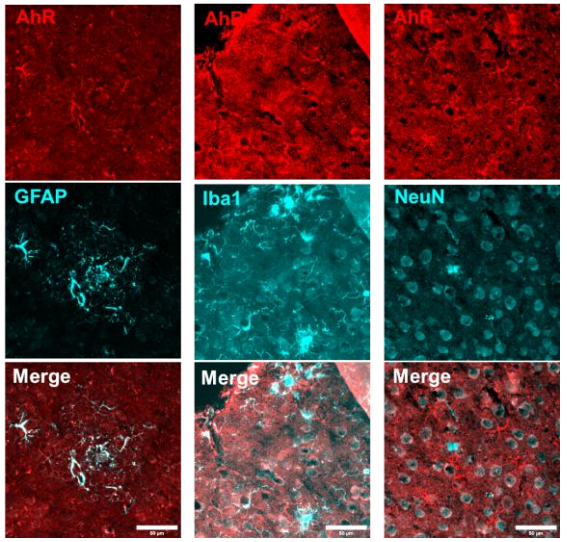
Kawajiri K et al. Exp Anim 2017

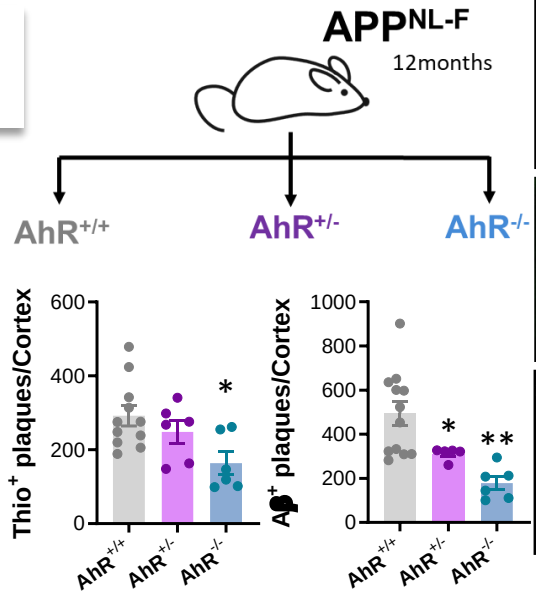
Microglial AhR drives Alzheimer's disease progression

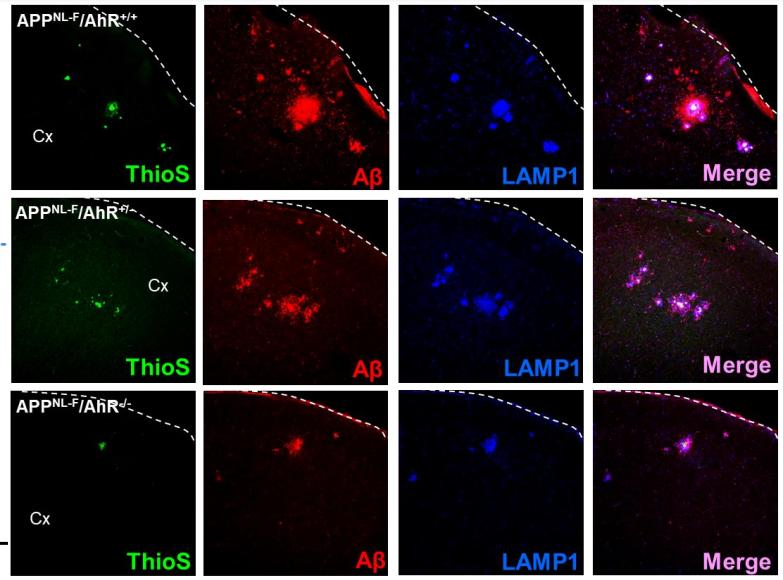


AhR expression is associated to **glial cells**

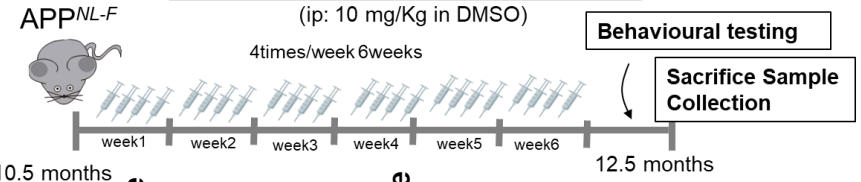


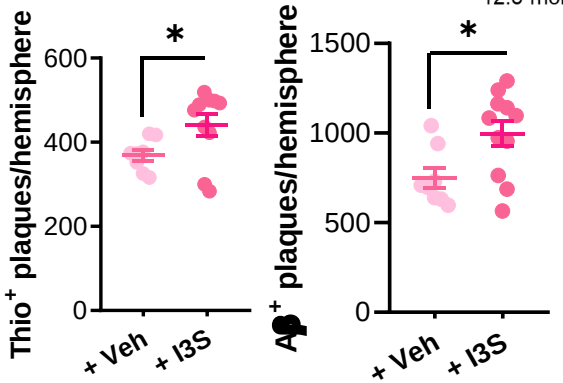


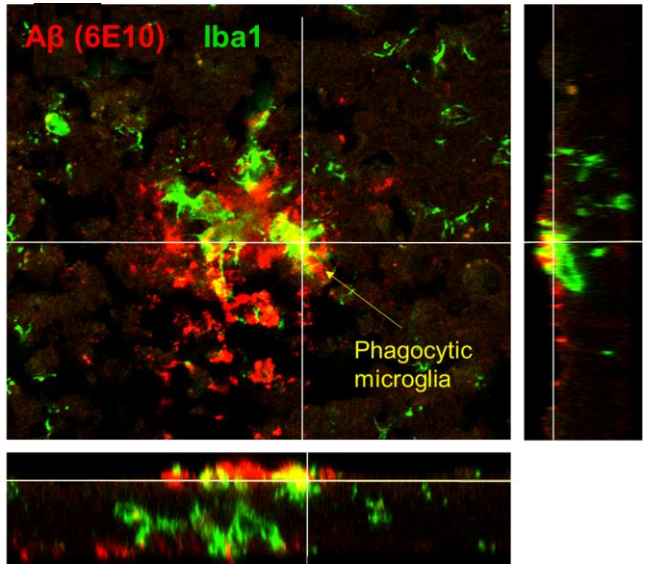




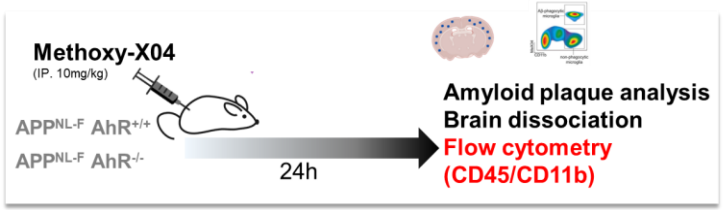
Chronic I3S administration

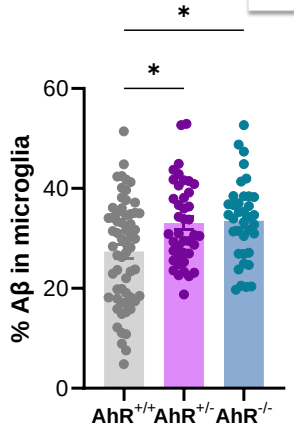


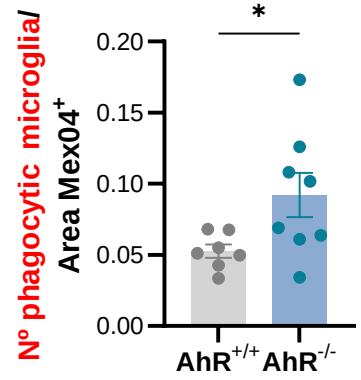




In vivo assay of phagocytosis

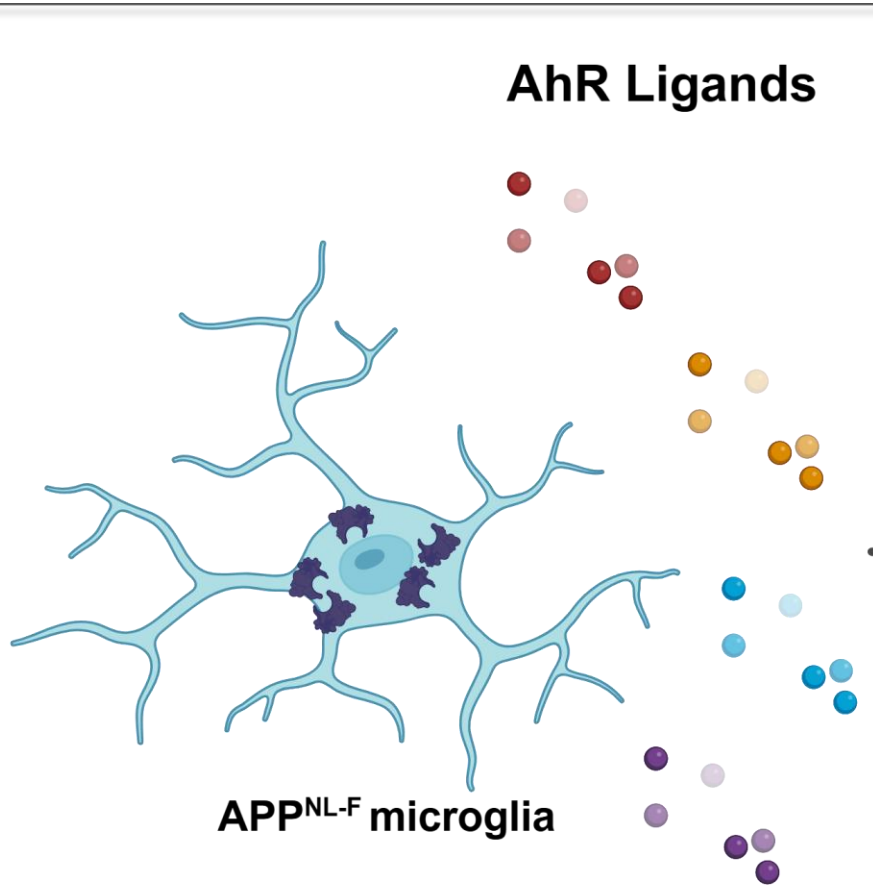




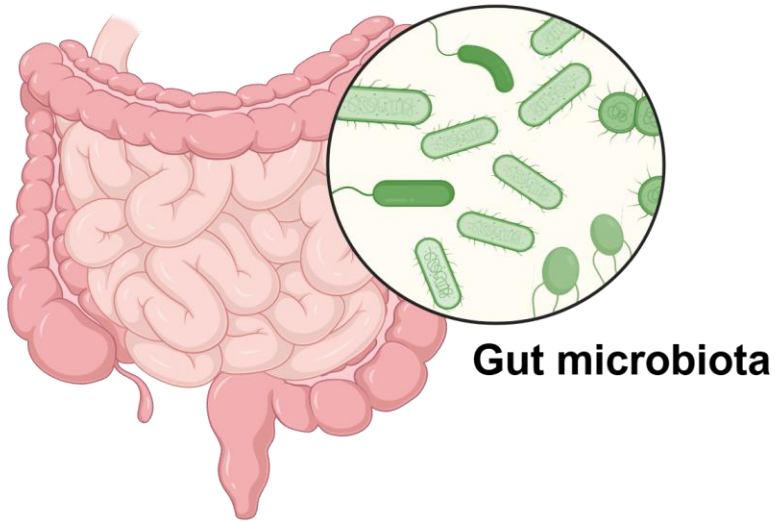


Nieto-Vaquero *and cols*. Unpublished data

How does AhR signaling modulate microglial function?

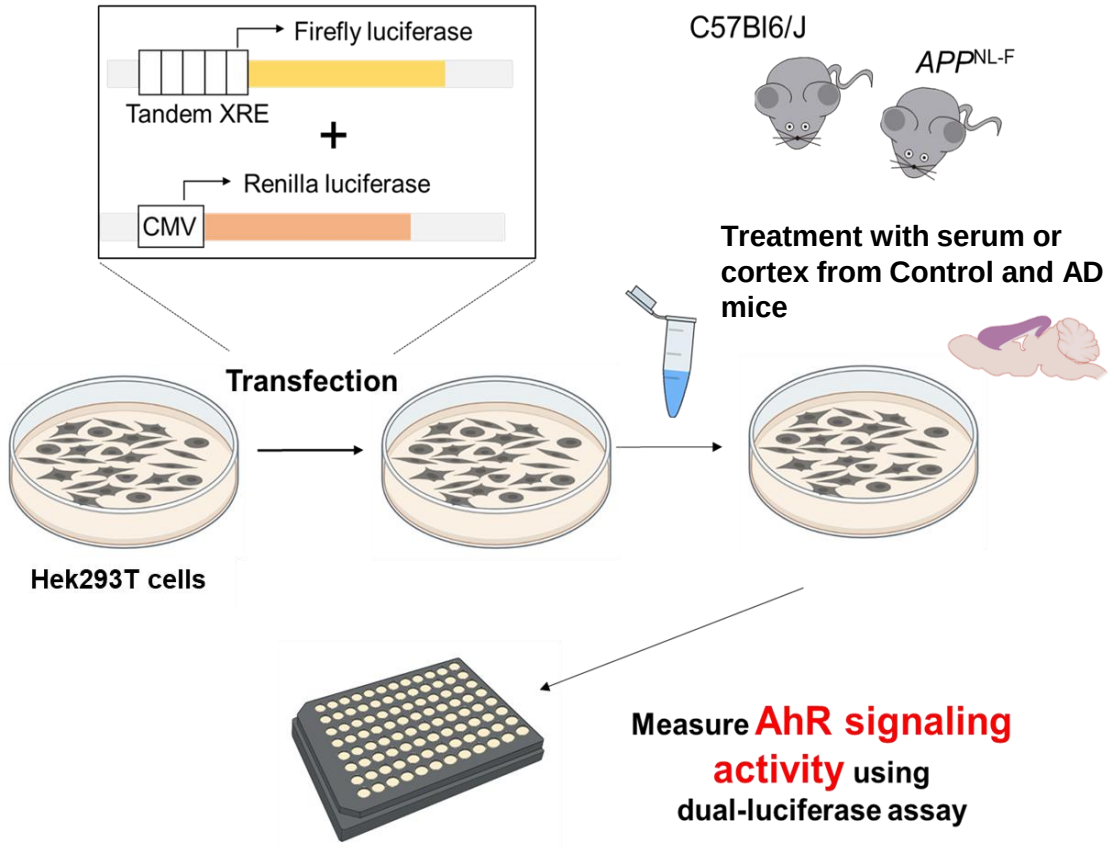


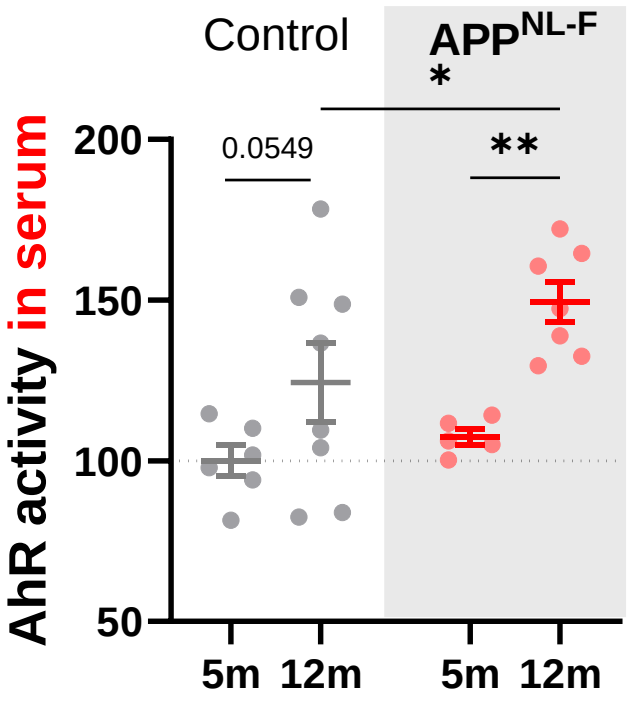
↑↑ AhR expression associated with amyloid plaque development

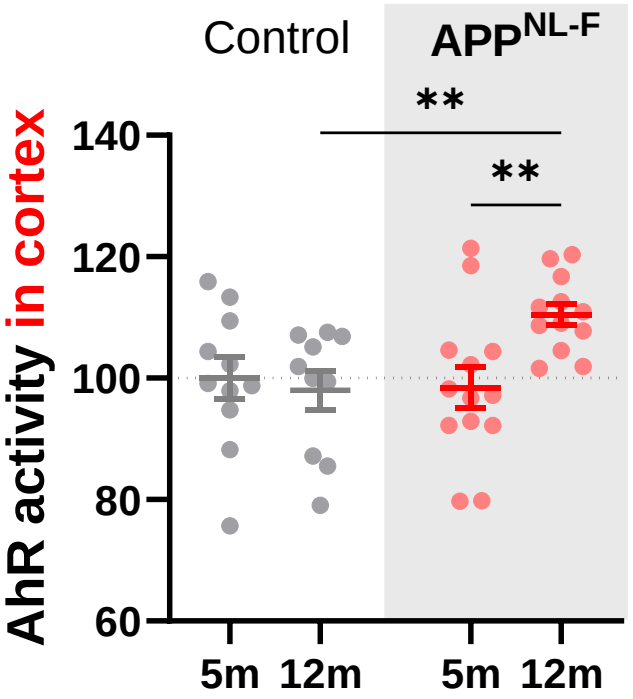


Are AhR ligands increased in Alzheimer's disease??

AhR ligands levels change with both aging and AD







Nieto-Vaquero *and cols*. Unpublished data

*p < 0.05 ; n = 5-14

Measuring AhR activity in AD patients

Colaboration with Dr. Alberto Villarejo Galende

Hospital Universitario 12 de Octubre

AhR Activity and metabolomics

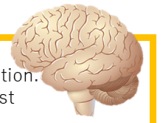
Blood and LCR Samples

- Glucemia
- Cholesterol
- HDL and LDL
- triglicerids
- 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

Imaging Study

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

Cognitive Study



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

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

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

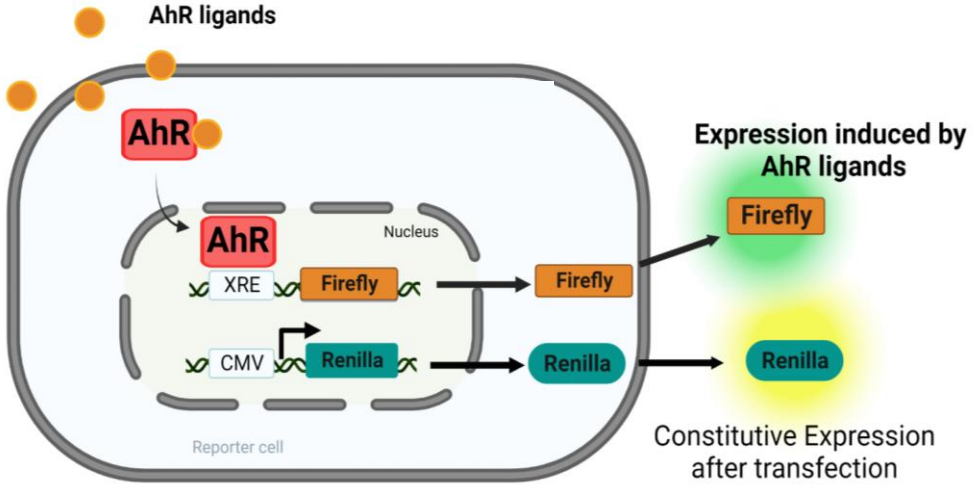
Ongoing

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.

Study Data

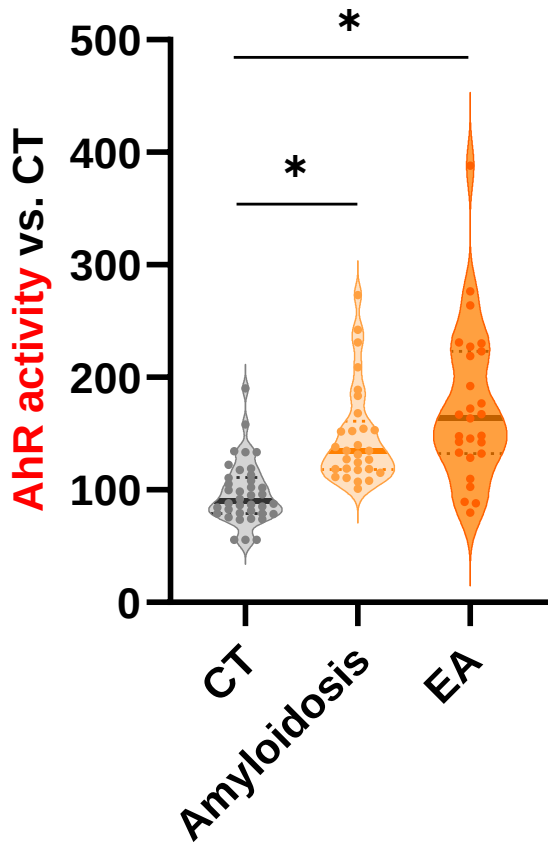
Clinical and demographic data
Predisposing factors. APOE genotype
Age, time of evolution of the disease and years of education
Sex; presence or absence of vascular risk factors (smoking, arterial hypertension, diabetes, mellitus, hypercholesterolemia
MEDAS (Mediterranean Dietary Adhesion Screener).



Aβ-/p-tau- → CT

Aβ+/p-tau- → Amyloidosis

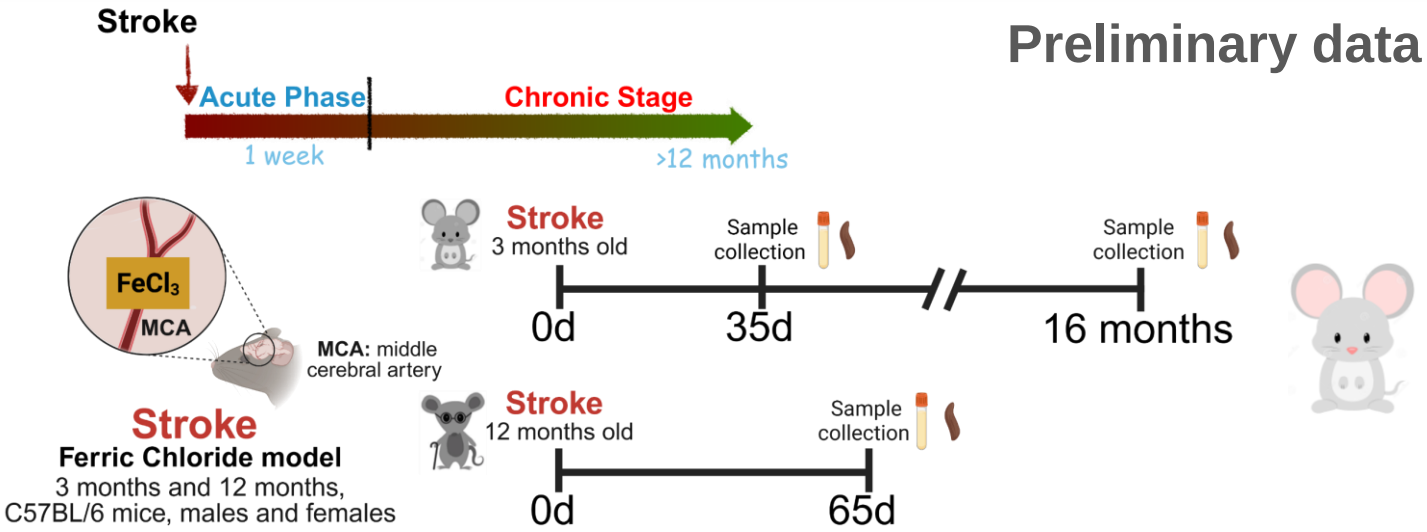
Aβ+/p-tau+ → AD

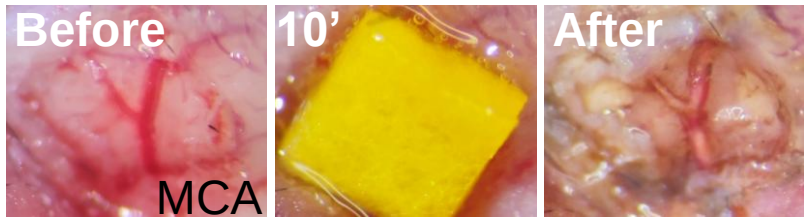


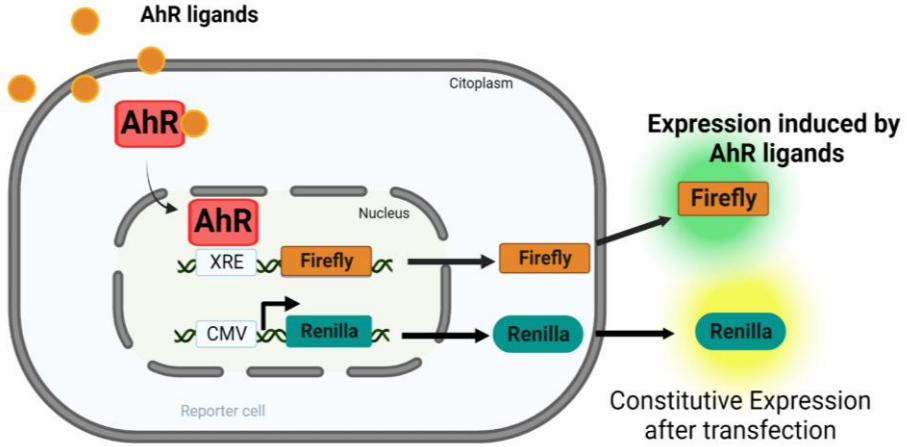
Nieto-Vaquero *and cols*. Unpublished data

*p < 0.05 *vs*. CT; n = 27-38

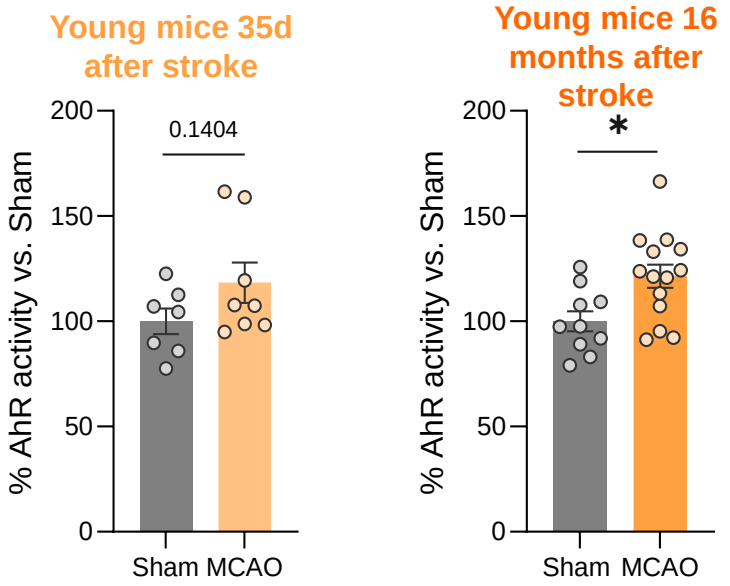
AhR ligands levels change in mice after stroke

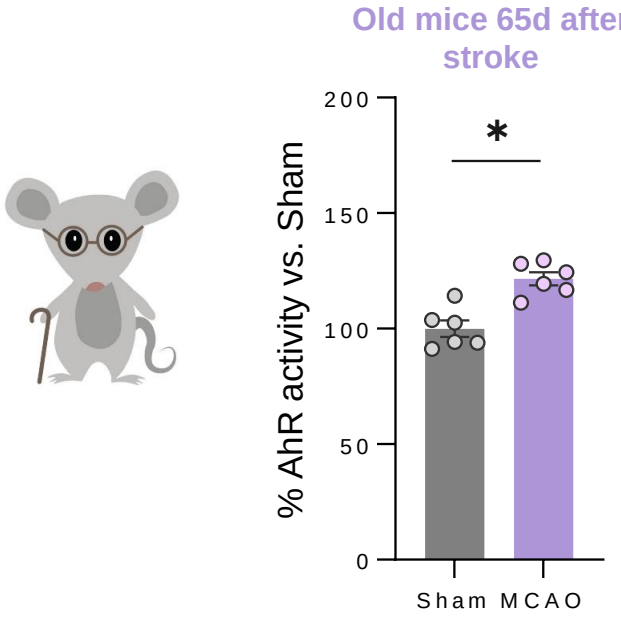






Preliminary data





Nieto-Vaquero *and cols*. Unpublished data

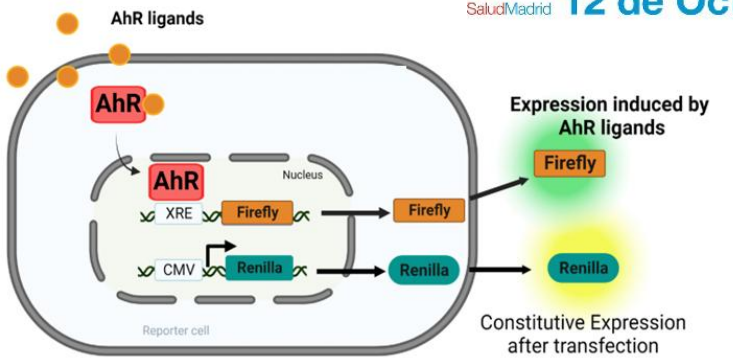
*p<0.05 vs APP^{NL-F} AhR$^{+/+}$ n=6-8

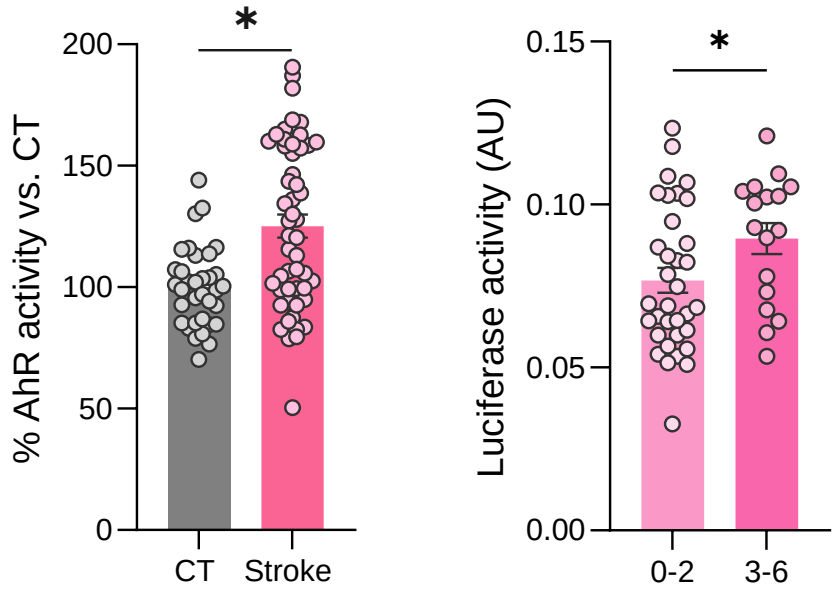
AhR ligands levels change in patients after stroke

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 Rakin Scale 3 months after stroke

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

Nieto-Vaquero *and cols*. Unpublished data

*p<0.05 vs CT or 0-2 n=35-55

Proyectos colaborativos para investigadores emergentes

Instituto de Salud Carlos III
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES
Cofinanciado por la Unión Europea

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

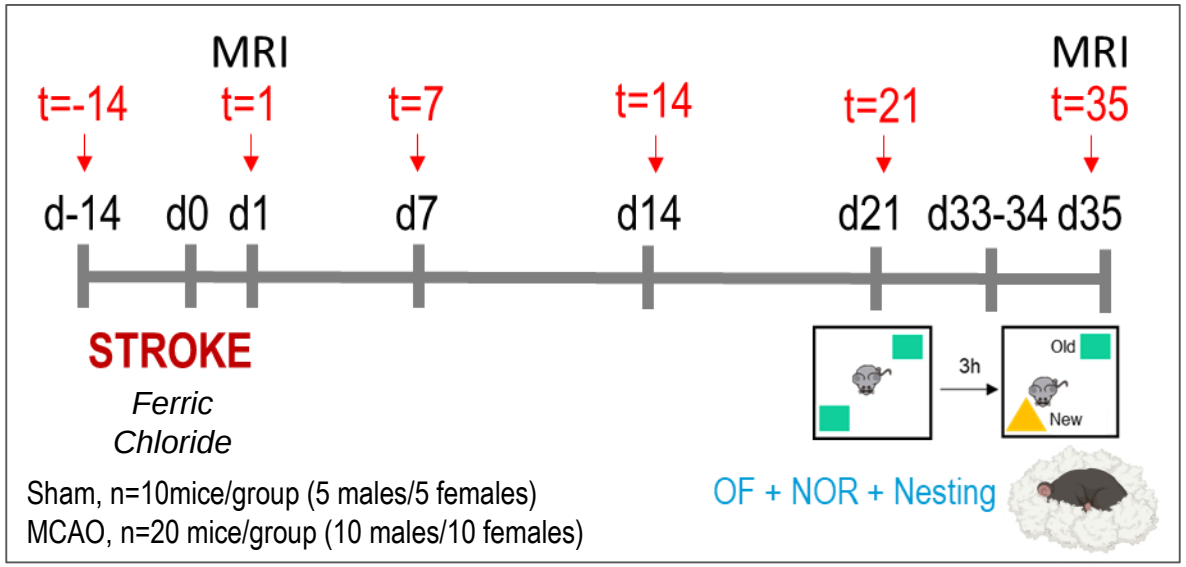
To clarify the **relationship** between **AhR ligands and post-stroke cognitive impairment** by analyzing these ligands in **animal models and in patient samples.**

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.**

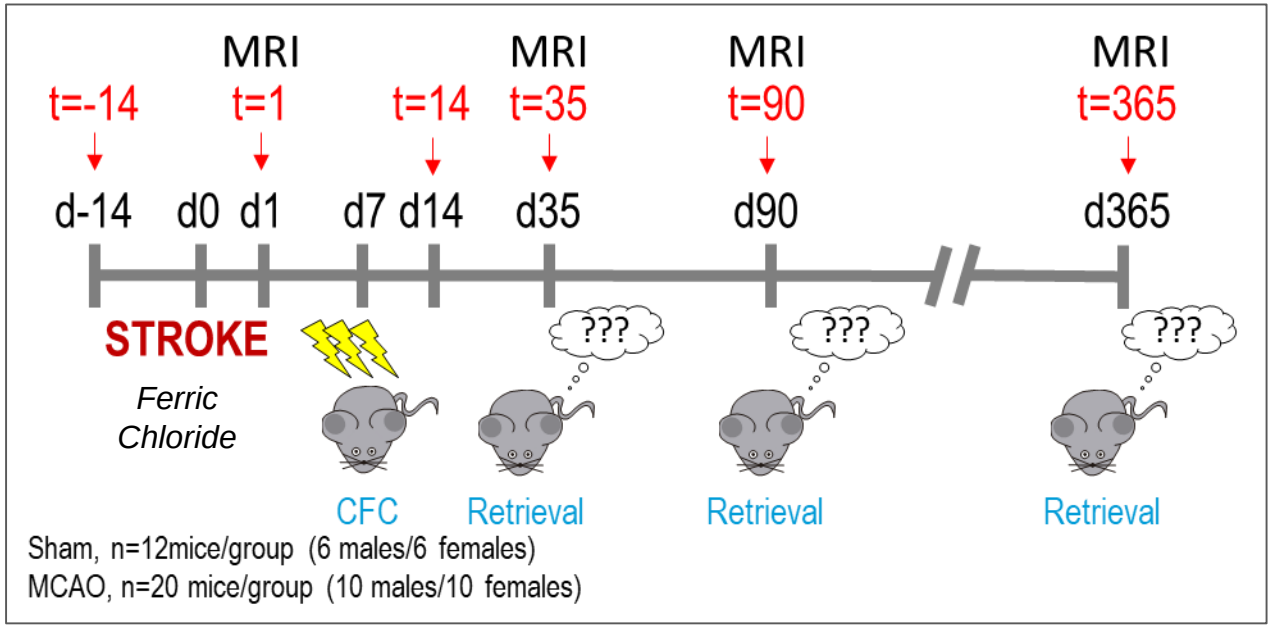
Characterization of AhR ligands in preclinical models of stroke

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

Ongoing analysis

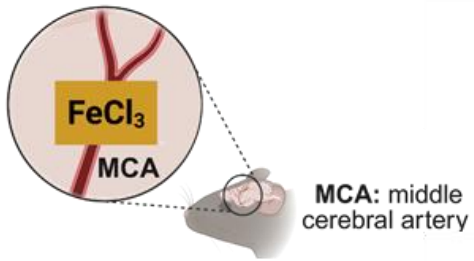


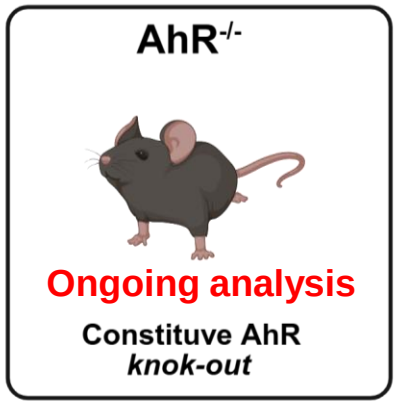
Sham, n=10mice/group (5 males/5 females)
MCAO, n=20 mice/group (10 males/10 females)

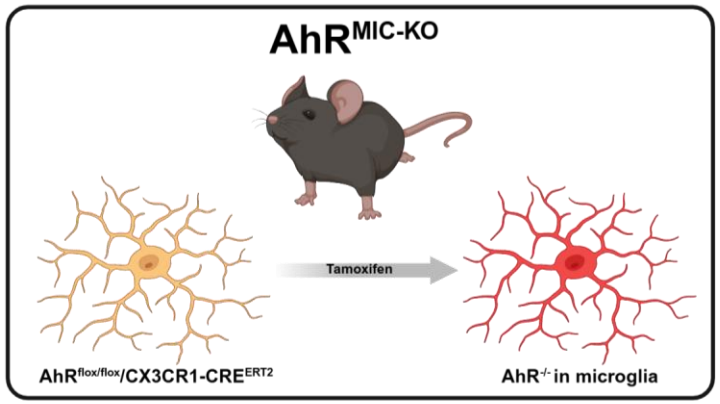


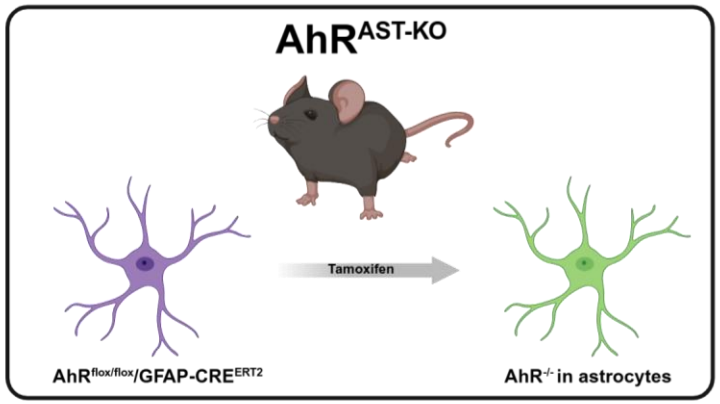
Sham, n=12mice/group (6 males/6 females)
MCAO, n=20 mice/group (10 males/10 females)

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





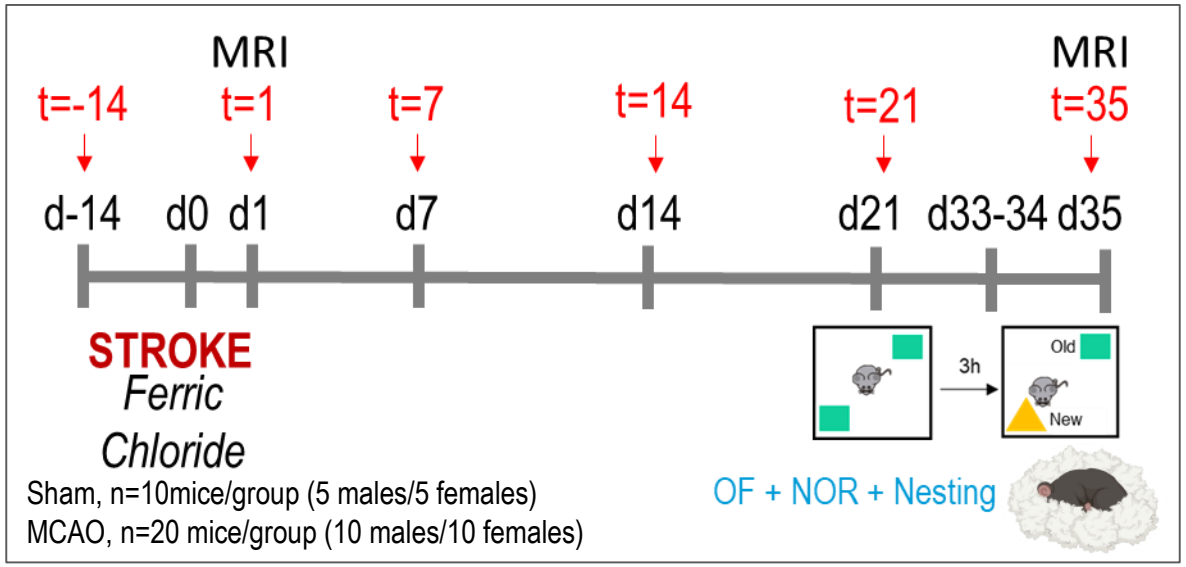




Characterization of AhR ligands in preclinical models of stroke

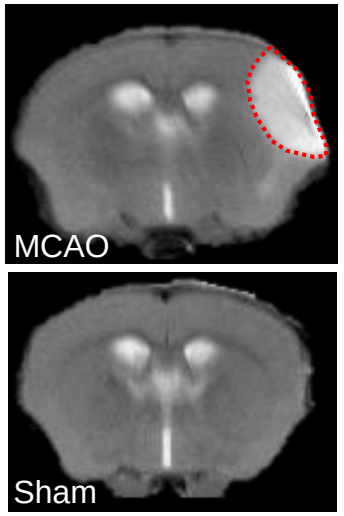
1. To evaluate AhR ligands in an animal model of ischemic stroke

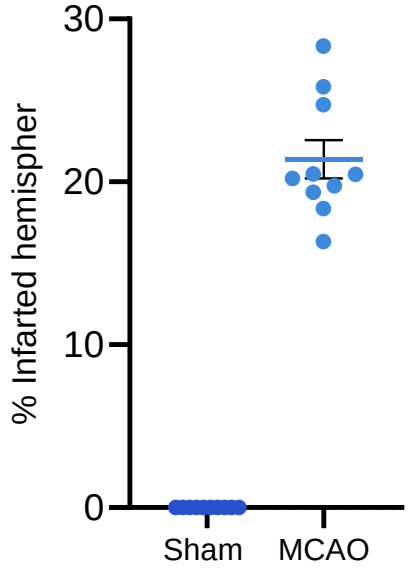
Ongoing analysis



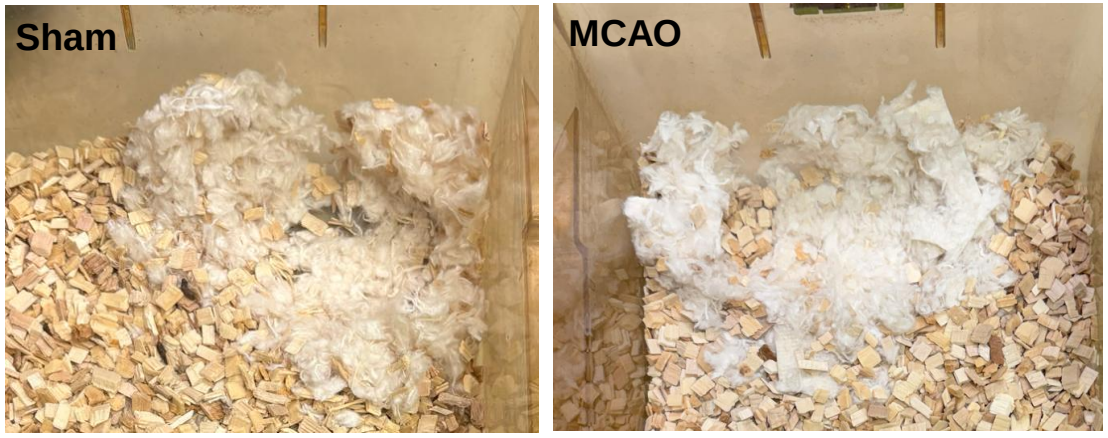
Sham, n=10mice/group (5 males/5 females)
MCAO, n=20 mice/group (10 males/10 females)

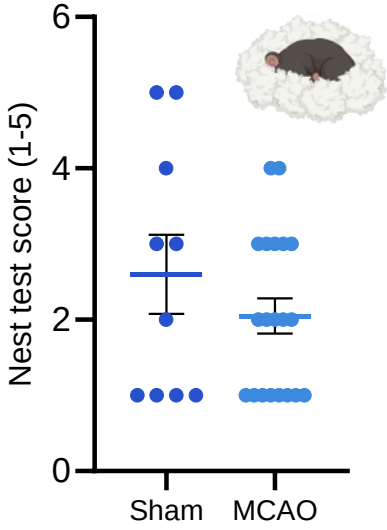
MRI 24h





Nesting test 35d



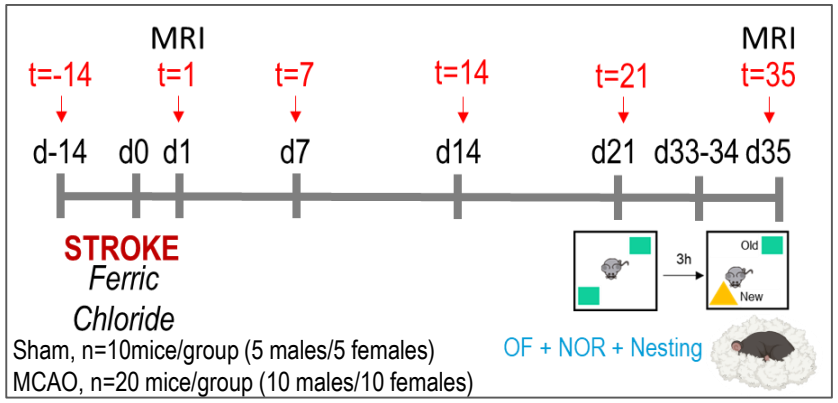


Carlos Parra (Surgeries), Virginia García (Behaviour test), Laura Martínez (Imaging)

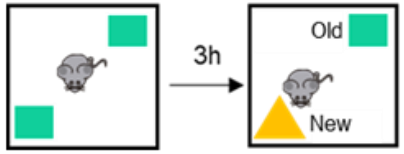
Characterization of AhR ligands in preclinical models of stroke

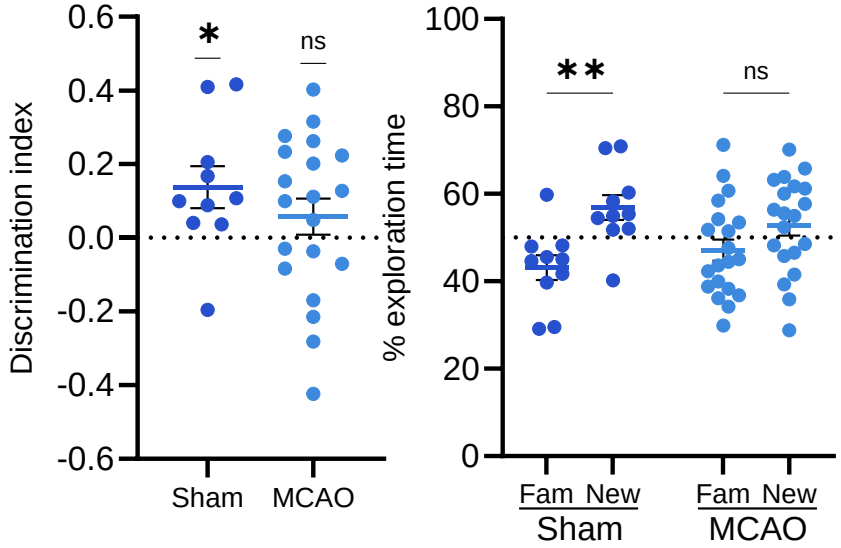
1. To evaluate AhR ligands in an animal model of ischemic stroke

Ongoing analysis

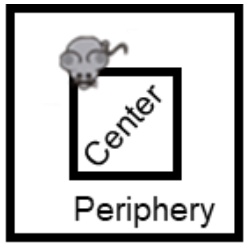


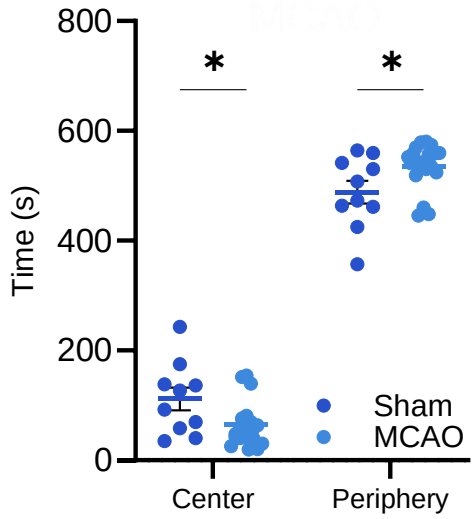
Novel object recognition test 35d

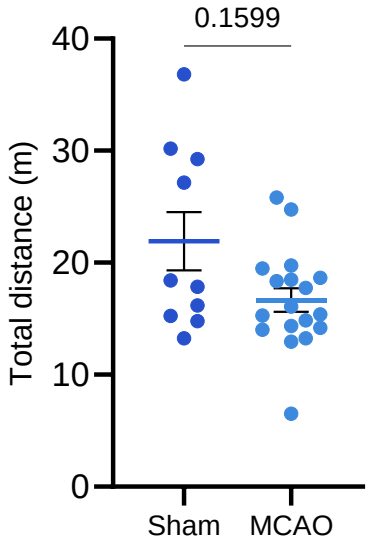


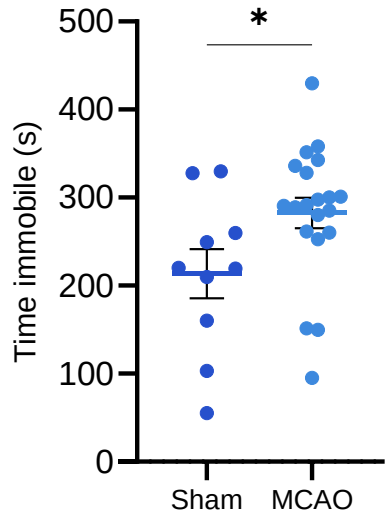


Open field 35d









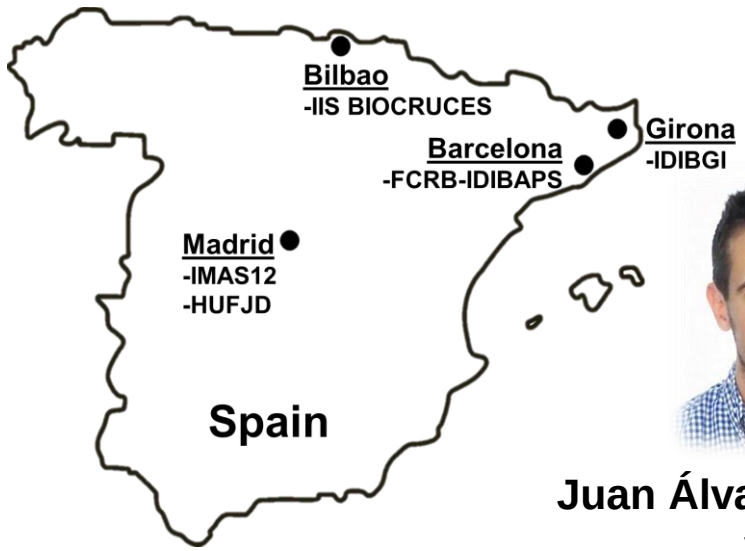
Ongoing:
-MRI analysis 35d post-stroke
-AhR ligands activity in serum, feces and brain
-Correlate AhR ligands-Cognitive impairment

*p<0.05 vs 0, Fam or Sham n=10-20

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

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)

i+12 Instituto de Investigación Hospital 12 de Octubre

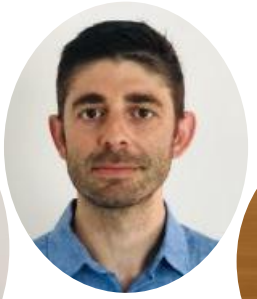
cnic



Maribel Cuartero (Ignacio Lizasoain y María Ángeles Moro)

IDIBAPS Institut D'Investigacions Biomèdiques August Pi i Sunyer

FUNDACIÓ CLÍNIC BARCELONA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Grupo quirónsalud

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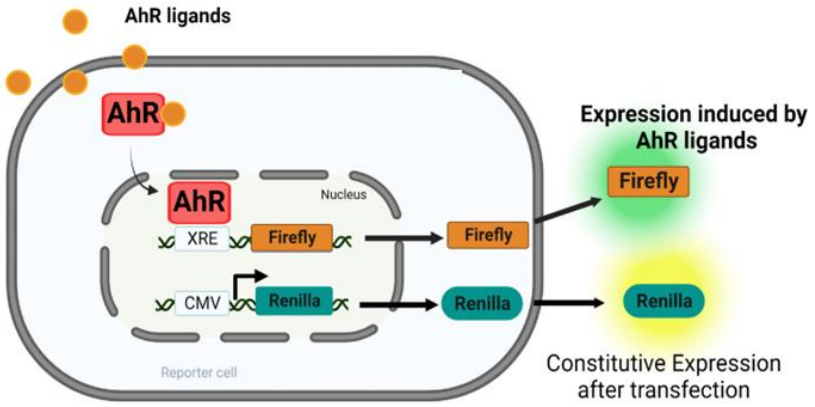
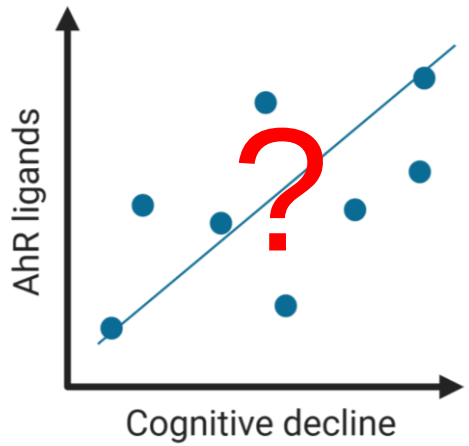
Evaluation of circulating AhR ligands in stroke patients

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





ALL OF YOU ARE INVITED TO PARTICIPATE!!

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Instituto de Salud Carlos III
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES
Cofinanciado por la Unión Europea

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