PhD thesis
NEUROSCIENCE
2019

Interleukin-6 trans-signaling in Alzheimer's disease

A study based on the Tg2576 and 3xTg-AD mouse models

ANNA ESCRIG MONFORT

Institute of Neurosciences
Department of Cellular Biology, Physiology and Immunology
Universitat Autònoma de Barcelona, Spain

Director

JUAN HIDALGO PAREJA

INc
Institut de
Neurociències

BCFI
Departament de
Biologia Cel·lular, Fisiologia i Immunologia
Universitat Autònoma de Barcelona

UAB
Universitat Autònoma de Barcelona

Institut de Neurociències
Facultat de Biociències
Departament de Biologia Cel·lular, Fisiologia i Immunologia
Universitat Autònoma de Barcelona

Interleukin-6 trans-signaling in Alzheimer's disease: A study based on the Tg2576 and 3xTg-AD mouse models

Anna Escrig Monfort
2019

Memòria de tesis presentada per **Anna Escrig Monfort** per tal d'optar al grau de Doctor en Neurociències per la Universitat Autònoma de Barcelona.

Aquest treball s'ha realitzat sota la dirección del Doctor **Juan Hidalgo Pareja**, catedràtic d'universitat del Departament de Biologia Cel·lular, Fisiologia i Immunologia i del Institut de Neurociències de la Universitat Autònoma de Barcelona.

Director de Tesis   Doctoranda

Dr. Juan Hidalgo Pareja   Anna Escrig Monfort

El viaje no termina jamás. Solo los viajeros terminan. Y también ellos pueden subsistir en memoria, en recuerdo, en narración… el objetivo de un viaje es solo el inicio de otro viaje.

J. Saramago

Als meus pares.

Index

ABSTRACT

Alzheimer's disease (AD) is the most common diagnosed dementia. Many scientific advances have been performed in order to understand AD pathogenesis and aethiology, however, no curative treatment has been discovered. AD courses with a progressive loss of cognitive functions, memory and language. It is characterized by the aggregation of β-amyloid peptides (Aβ) forming extracellular "plaques" and hyperphosphorylated tau protein forming intracellular neurofibrillary "tangles" (NFTs). These changes are accompanied by neuronal loss, microvascular damage and pronounced neuroinflammation.

IL-6 is a pleiotropic cytokine which has an important role in neuroinflammatory processes. It has been suggested that it could be involved in the aethiology of AD. It has a complex signaling pathway mediated via three distinct modes. (1) Classic signaling involves the binding of a molecule of IL-6 to a specific membrane receptor (mIL-6R), which activates dimerization of gp130 and subsequent downstream JAK/STAT signaling; (2) in the trans-signaling pathway, IL-6 binds to a soluble form of mIL-6R (sIL-6R) to activate gp130. Due to the ubiquitous expression of gp130, most cells in the body can be stimulated by this process. IL-6 trans-signaling is specifically inhibited by the soluble form of gp130, sgp130. (3) Cluster signaling, which has been described as an exclusive mechanism for the communication between dendritic and T cells.

As many of the detrimental effects of IL-6 in the CNS occur via trans-signaling, in this thesis we have characterized the role of IL-6 trans-signaling in AD, by crossing transgenic mice expressing sgp130Fc (specific inhibitor of IL-6 trans-signaling) in astrocytes with two different mouse models of AD, the Tg2576 and the 3xTg-AD mice. The Tg2576 had a more robust AD phenotype what facilitated the identification of the effects of the inhibition of IL-6 trans-signaling, which modulated survival, behavioral traits and spatial learning and memory in a sex-dependent manner. Blocking IL-6 trans-signaling decreased amyloid plaques and/or $A\beta_{40}$ and $A\beta_{42}$ levels in the cortex of female mice of both AD models. The changes elicited on gliosis by this blocking were unrelated to the amyloid cascade.

Middle-age obesity is a risk factor for AD and besides, many AD patients have to cope with metabolic alterations and loss of body weight when the disease is already present. Since IL-6 is an important modulator of metabolism and energy balance, the role of IL-6 trans-signaling in metabolism and body weight have also been analyzed in the 3xTg-AD model under control and high-fat diet (HFD) conditions. 3xTg-AD animals presented a hypermetabolic state, which was upregulated by the inhibition of IL-6 trans-signaling in females after HFD, whereas the opposite trend was observed in males; endocrine and metabolic changes were in agreement with these effects. Surprisingly, Aβ levels were diminished after the HFD treatment in comparison with mice fed with a normal chow control diet.

These results suggest that IL-6 trans-signaling is involved in the pathogenesis of AD in these two mouse models; it should be considered as a potential therapeutic target for the creation of new AD treatments.

ABBREVIATIONS

A β	β-amyloid peptide
AD	Alzheimer's disease
ADAM	A disintegrin and metalloproteinase
Agrp	agouti-related protein
AICD	APP intracellular domain
APLP	APP-like protein
APOE	apolipoprotein E
APP	amyloid precursor protein
ARRIVE	Animal Research: Reporting of *In Vivo* Experiments
ATM	adipose tissue macrophages
BACE	β-side APP-cleaving enzyme
BAT	brown adipose tissue
BBB	blood-brain barrier
BSA	bovine serum albumin
BSF2	B-cell differentiation factor 2
CAA	cerebral amyloid angiopathy
CBF	cerebral blood flow
CDKN2A	cyclin-dependent kinase inhibitor 2A
CHR	cytokine receptor homology region
CKK	cholecystokinin
CLC	cardiotrophin-like cytokine
CNS	central nervous system
CNTF	ciliary neurotrophic factor
CSF	cerebrospinal fluid
CT-1	cardiotrophin 1
CTF	C-terminal fragment
CTX	cortex
DAB	diaminobenzidine
DAM	disease-associated microglia
DAMPs	danger-associated molecular patterns
DE	differentially expressed
DNA	deoxyribonucleic acid
EGF	endothelian growth factor
ELISA	enzyme-linked-immuno-sorbent assays
EOA	entries open arms
EOFAD	early-onset familial Alzheimer's disease
EPM	elevated-plus maze
F&R	fasting and refeeding test
FDA	Food and Drug Administration
FDG	fluorodeoxyglucose

FGF-21		fibroblast growth factor 21
FI		frailty index
FTLD		frontotemporal lobar degeneration
Gapdh		glyceraldehyde 3-phosphate dehydrogenase
GEE		generalized estimated equations
GFAP		glial fibrillary acidic protein
GLP-1		glucagon-like-peptide-1
GLZ		generalized linear model
gp130		glycoprotein 130
	sgp130	soluble glycoprotein 130
GWAS		genome-wide association study
HB		hole-board
HD		head-dipping
HFD		high-fat diet
HGF		hybridoma growth factor
HPA		hypothalamic-pituitary-adrenal (axis)
HPC		hippocampus
HSF		hepatocyte stimulating factor
IBA-1		ionized calcium-binding adaptor protein-1
ICAM-1		intracellular adhesion molecule-1
ICV		intracerebroventricular
IDE		insulin degrading enzyme
IGF		insulin-like growth factor
IHQ		immunohistochemistry
IL		interleukin
IL-6R		Interleukin-6 receptor
	mIL-6R	membrane IL-6 receptor
	sIL-6R	soluble IL-6 receptor
INF-β2		interferon-β2
ITT		insulin tolerance test
JAK		janus kinase
LIF		leukaemia inhibitory factor
LOAD		late-onset Alzheimer's disease
LPBN		lateral parabrachial nucleus
LPS		lipopolysaccharide
Mac1		macrophage-1 antigen
MAPK		mitogen-activated protein kinase
MRI		magnetic resonance imaging
MWM		Morris water maze
NEP		neprilysin
NFL		neurofilament light
NFTs		neurofibrillary tangles
NO		nitric oxide

NORT	novel object recognition test
NSE	neuron-specific enolase
NSPCs	neural stem or progenitor cells
OF	open field
OGTT	oral glucose tolerance test
PAMPs	pathogen-associated molecular patterns
PAXIP1	PAX-interacting protein
PEN	presenilin enhancer
PEPCK	Phosphoenolpyruvate carboxykinase
PET	positron emission tomography
PFA	paraformaldehyde
PI3K	phosphatidyl-inositol-3-kinase
PIAS	protein inhibitor of activated STAT
PSEN	presenilin
PTZ	pentylenetetrazole
RNA	ribonucleic acid
ROS	reactive oxygen species
SH2	src homology 2
SHP2	SH2 domain-containing phosphatase 2
SOCS3	suppressor of cytokine signaling 3
STAT	signal transducer and activator of transcription
TBI	traumatic brain injuries
TCR	T-cell receptor
TLR	Toll-like receptors
TNF	tumor necrosis factor
TOA	time open arms
TQ	target quadrant
TYK	tyrosine kinase
VCAM-1	vascular cell adhesion molecule-1
VNTR	variable number tandem repeat
WAT	white adipose tissue
WB	western blot

GENERAL INTRODUCTION

Interleukin-6 (IL-6)

Introduction

Cytokines are small secreted proteins which are engaged in communication and interaction between cells. They may act on the same cells that previously secreted them (autocrine action), on nearby cells (paracrine action) or on distant cells (endocrine action). Cytokines are characterized for having redundant functions, since similar functions can be done by different cytokines, and for having pleiotropy as one cytokine can act on several cell types. The word "cytokine" is used as a general name and encompasses many different proteins. Cytokines made by a leukocyte and acting on other leukocytes were named interleukins (Zhang and An 2009).

Interleukin-6 (IL-6) is a pleiotropic cytokine that was originally identified in 1985 as a B-cell differentiation factor (BSF-2) that stimulated the maturation of B cells to antibody-producing cells (Hirano et al. 1985). IL-6 DNA was isolated in 1987 by the same group (Yasukawa et al. 1987). Different laboratories gave different names to this protein such as interferon-β2 (INF-β2) or hepatocyte stimulation factor (HSF); to avoid confusing nomenclature, this factor was renamed to "interleukin-6" (Poupart et al. 1987; Yasukawa et al. 1987; Schaper and Rose-John 2015).

Both human and murine IL-6 genes have been isolated and mapped. Human *IL-6* gene maps to chromosome 7 (Sehgal et al. 1986), consists of five exons and four introns and encodes 212 amino acids (Yasukawa et al. 1987). Murine *Il-6* maps to chromosome 5, it also has five exons and four introns and encodes 211 amino acids.

Different classification methods have been created in order to categorize the large number of cytokines discovered during the past years. Cytokines can be classified depending on (1) their structure such as helical cytokines, the trimeric tumor necrosis factor (TNF) family, the cysteine knot growth factors or the β-trefoil growth factors; (2) according to the receptor they bind as class I cytokine receptors, class II cytokine receptors, TNF receptors, tyrosine kinase receptors, and chemokine receptors; (3) depending on their physiological role, in this sense, IL-6 is the founding member of neuropoietin family for its effects on hematopoietic and nervous system; (4) the sharing of a receptor subunit, IL-6 is comprised in the gp130 family (Erta et al. 2012), which besides, includes other structurally related cytokines as IL-11, IL-27, leukaemia

inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotrophin 1 (CT-1), neuropoietin and cardiotrophin-like cytokine (CLC) (Bauer et al. 2007).

Structurally, IL-6 protein is a four-helix bundle cytokine arranged in an up-up-down-down topology (helices A and B are arranged in the same direction, whereas C and D in the opposite, a long loop links A with B, then, a short one linking B and C and, finally another long loop between C and D) (Somers et al. 1997). Human IL-6 range from 23 to 30 kDa, while murine range from 22-29 kDa, the differences in weight are due to post-translational modifications as glycosylation or phosphorylation (May et al. 1988a, b).
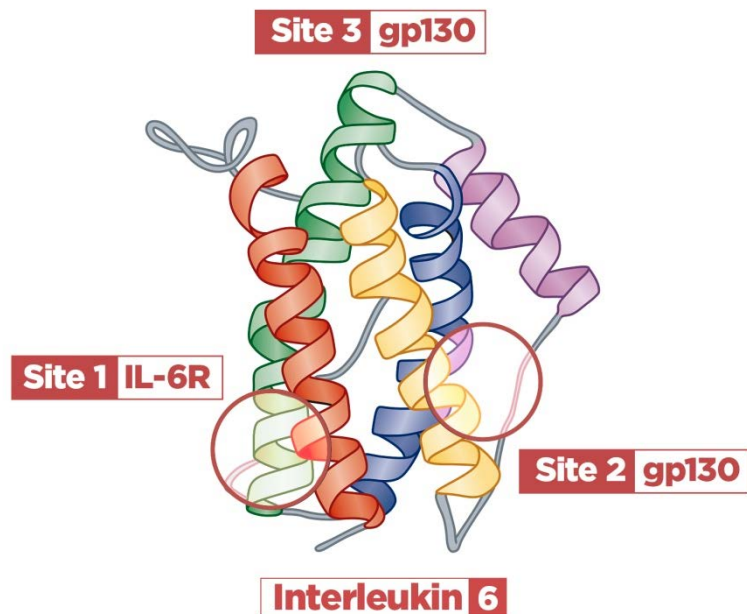
Figure 1. Structure of IL-6. The four up-up-down-down helices and the binding site for IL-6R (site 1) and for gp130 (site 2 and 3). Adapted from (Hunter and Jones 2015).

IL-6 signal transduction

The IL-6R complex consists of two polypeptides: the α-chain (IL-6R or CD126) which is a 80 kDa transmembrane glycoprotein without intrinsic signaling activity and the β-chain (gp130 or CD130), a 130 kDa transmembrane protein that binds IL-6/IL-6R and it is required for signal transducing (Taga et al. 1989).

IL-6R is a class I cytokine receptor cloned in 1988 (Yamasaki et al. 1988). It forms an Ig-like domain 100 amino acids from the N-terminal domain; the ligand interaction site (cytokine receptor homology region, CHR) is composed by two fibronectin type III modules characterized by conserved cysteine residues and a Tryptophan-Serine-X-Tryptophan-Serine (WSXWS) motif above the transmembrane portion (Bazan 1990). The intracytoplasmic region of the receptor is very short and lack any known signal transduction motif (i.e. tyrosine- or serine/threonine kinase domain) (Kishimoto et al. 1995).

As stated above, IL-6R requires the oligomerization with gp130 to signal (Murakami et al. 1993). This association occurs extracellularly and in presence of IL-6, in fact, human and murine IL-6R show high homology in the extracytoplasmic regions indicating that this region plays an important role (Taga et al. 1989). Structurally, gp130 has six domains: an N-terminal Ig-like domain (D1), a cytokine-binding module (CHR, D2–D3) characterized also by conserved cysteine residues and a Tryptophan-Serine-X-Tryptophan-Serine (WSXWS) motif, and three contiguous, membrane-proximal fibronectin type III modules (D4–D6) (see Figure 2) (Xu et al. 2010).

From a ligand point of view, soluble IL-6 binds IL-6R through site 1, forming a heterodimer. Secondly, this heterodimer binds gp130 through site 2. Thirdly, two heterotrimers (IL-6/IL-6R/gp130) join by interactions in site 3 (Somers et al. 1997). But, the stoichiometry of the signaling complex is still debated and different compositions has been proposed for the interactions between these three factors: tetrameric IL-6/IL-6R/(gp130)$_2$ (Grötzinger et al. 1997) or hexameric (IL-6)$_2$/(IL-6R)$_2$/(gp130)$_2$ (Ward et al. 1994; Paonessa et al. 1995). A mixed model was proposed (Grötzinger et al. 1999), two gp130 molecules would contact each other via their CHR and Ig-like domain. When IL-6/IL-6R bound to a preformed gp130 homodimer, a tetrameric complex will assemble. However, due to the symmetry of the original gp130 homodimer the tetramer would still be able to bind another IL-6/IL-6R resulting in a hexameric complex. Low concentrations of IL-6/IL-6R might lead to the formation of active tetramers, whereas higher concentrations would shift the tetramer into an inactive hexamer.

In both cases, the dimerization of gp130 is needed for signal transduction (see Figure 2). The first enzymatic step that takes part in this process is the activation of Janus Kinases (JAK1, JAK2 and TYK2)(Stahl et al. 1994). Activated JAK kinases

phosphorylate tyrosine motifs within the cytoplasmic region of gp130 which act as recruitment sites for signaling components containing S̲rc h̲omology 2̲ (SH2) domains. The five most membrane-distal tyrosine motifs (Y759, Y767, Y814, Y905, Y915) are recruitment sites for members from the family of s̲ignal t̲ransducers and a̲ctivators of t̲ranscription (STAT), STAT3 and STAT1 (Stahl et al. 1995; Gerhartz et al. 1996). After recruitment, STAT proteins become phosphorylated in tyrosine residues, then, homo- or heterodimerize and translocate into the nucleus to regulate the transcription of IL-6-inducible genes (Lütticken et al. 1994; Zhong et al. 1994).

Apart from the JAK/STAT signaling pathway, IL-6 also activates the m̲itogen-a̲ctivated p̲rotein k̲inase (MAPK) and p̲hosphatidyl-i̲nositol-3̲-k̲inase (PI3K) cascades. The phosphorylated Y759 residue recruits S̲H2̲-domain-containing p̲hosphatase 2̲ (SHP2) and promotes the translocation of cytoplasmic Gab1 to the membrane. Gab1 acts as a signaling platform for SHP2, Grb2, PI3K, phospholipase Cγ (PLCγ) and Ras-GTPase activating protein (Ras-GAP) (Eulenfeld and Schaper 2009). According to the current view SHP2-dependent activation of MAPK, SHP2 interacts with Grb2-SOS complex that allows Ras activation, and consequently, Raf and MAPK cascade (Fukada et al. 1996; Schiemann et al. 1997). Regarding the activation of PI3K/Akt cascade, the mechanism is still unclear, however, it is thought to be also activated after the stimulation of Gab1(Takahashi-Tezuka et al. 2015).

A balance between JAK/STAT and MAPK activation is necessary to avoid IL-6 overstimulation. In this sense, the phosphorylation of tyrosine Y759 (Y757 in mice) also plays an important role (Heinrich et al. 2003; Eulenfeld et al. 2012; Schaper and Rose-John 2015). As stated above, the phosphorylated Y759 motif recruits SHP2 initiating the MAPK cascade; additionally, SHP2 dephosphorylates tyrosine motifs of the gp130, reducing even more the STAT-mediated signaling. This residue also recruits the feedback inhibitor s̲uppressor o̲f c̲ytokine s̲ignaling 3̲ (SOCS3) which is a fast feedback inhibitor of the JAK kinases (Schmitz et al. 2000). Furthermore, the p̲roteins i̲nhibitors of a̲ctivated S̲TAT (PIAS) are important co-regulators of the JAK/STAT pathway; PIAS1 inhibits DNA binding of activated STAT1 and, therefore, STAT1-mediated gene expression (Liu et al. 1998), whereas, PIAS3 inhibits specifically STAT3 (Chung et al. 1997). The receptor internalization and degradation also contributes to the negative regulation of the signal transduction; the signal transducing receptor gp130 internalizes constitutively because its cytoplasmic tail includes a di-leucine internalization motif (L786, L787) (Dittrich et al. 1994). The internalization of gp130 is crucial for the ligand-induced internalization of the entire

IL-6R complex, and it is dependent of SOCS3 protein expression (Thiel et al. 2000). After internalization, the IL-6-stimulated receptors go into lysosomal degradation preventing, then, IL-6 overstimulation (Tanaka et al. 2008).
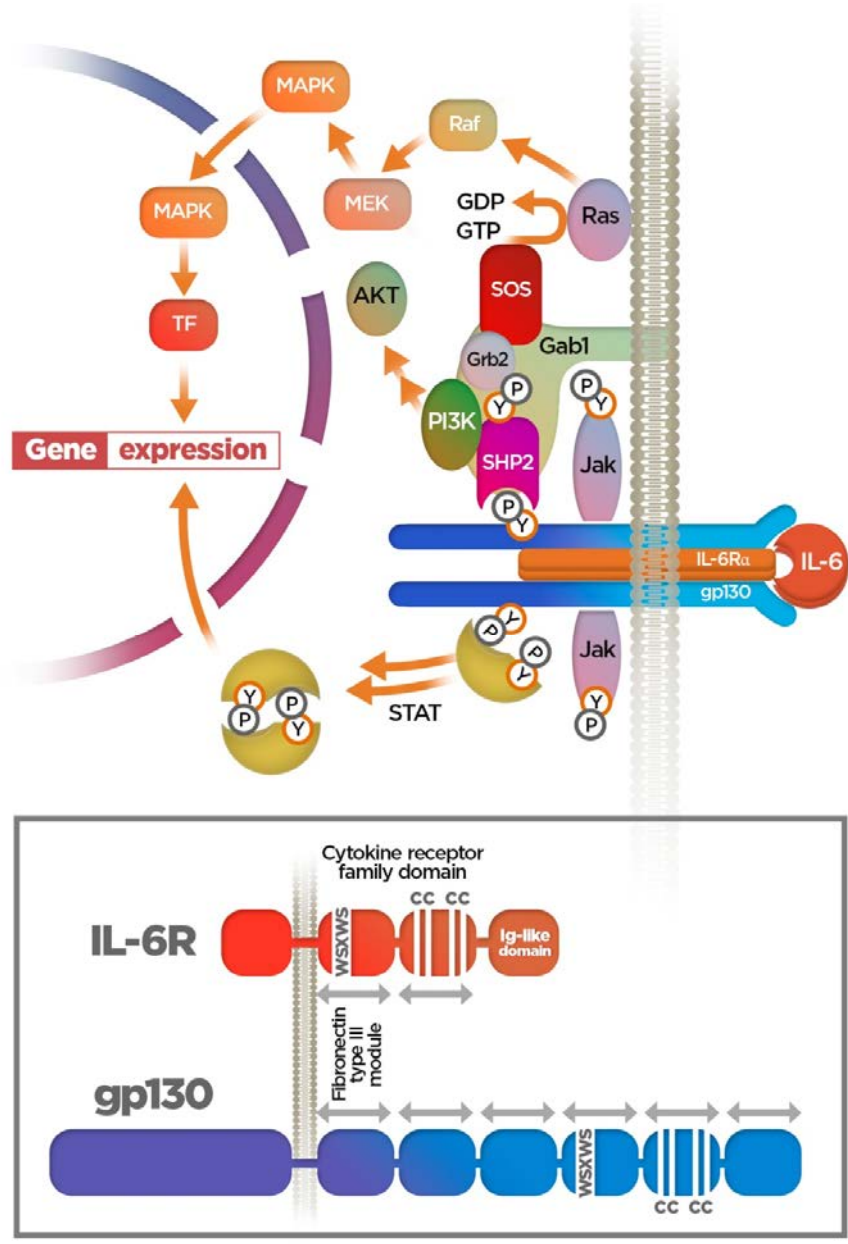

Figure 2. IL-6 intracellular signaling and and schematic structure of IL-6R complex. The activation of the STAT, MAPK and PI3K cascades contributes to the

regulation of IL-6-induced gene expression. Image adapted from (Kishimoto et al. 1995; Eulenfeld et al. 2012).

IL-6 signaling pathways: IL-6 trans-signaling

The mechanism described in the previous section is what is known as classic signaling pathway and involves an IL-6R anchored to the membrane (mIL-6R) and the signal transducer gp130 (Schaper and Rose-John 2015). In 1993 was demonstrated that a natural soluble form of IL-6R (sIL-6R) acts as an agonist together with its ligand (Mülberg et al. 1993). This phenomena is what is called trans-signaling (Schaper and Rose-John 2015) and it is IL-6-specific, since in other cytokines such IL-1 (Fanslow et al. 1990) or IL-4 (Fanslow et al. 1991), the soluble form of the receptor has antagonistic activity rather than agonistic. IL-6 bound to sIL-6R may activate gp130 in cells which do not express the mIL-6R. While gp130 is expressed on all cells of the human body, IL-6R expression is more limited to certain type of cells. Therefore, IL-6 trans-signaling widens the IL-6 effects.

To generate the soluble form of the receptor, in humans the mIL-6R can be proteolytically cleaved from the cell membrane (Mülberg et al. 1993) or generated by alternative splicing of the mRNA (Lust et al. 1992), whereas in mice only proteolysis has been confirmed. A disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) is a membrane bound metalloprotease and it is the main sheddase for human IL-6R. It cleaves IL-6R between amino acid positions Q357-D358, close to the transmembrane region (Müllberg et al. 1994). In addition to ADAM17, ADAM10 has also been implicated in sIL-6R production (Matthews et al. 2003), being the main sheddase in mice (Garbers et al. 2011). In fact, ADAM10 seems to be responsible of constitutive cleavage, whereas, ADAM17 is stimulated under pathological conditions (Matthews et al. 2003). Later on, two novel proteases were postulated as novel IL-6R sheddases, meprin α and meprin β (Arnold et al. 2017a).

A soluble form of gp130, sgp130, also exists naturally. It is a specific inhibitor of the IL-6 trans-signaling with no effects on the classical pathway (Jostock et al. 2001). The majority of data suggest that the soluble form is generated by alternative mRNA splicing (Diamant et al. 1997). However, gp130 shedding by proteases has not been excluded (Sherwin et al. 2002), but the responsible proteases remain to be determined (Wolf et al. 2014). Sgp130 is a competitive inhibitor; it binds IL-6/sIL-6R before they

become attached to the membrane, hence, a molar excess is required to antagonize IL-6 signaling.

Recently, a third signaling pathway has been described, the cluster signaling or trans-presentation. T cells responded to IL-6 in the absence of mIL-6R. IL-6/mIL-6R complex from dendritic cells is presented to gp130-expressing T cells, in combination with the T-cell receptor (TCR) signal. This mechanism is required for the priming of pathogenic T_H17 cells (Heink et al. 2016).
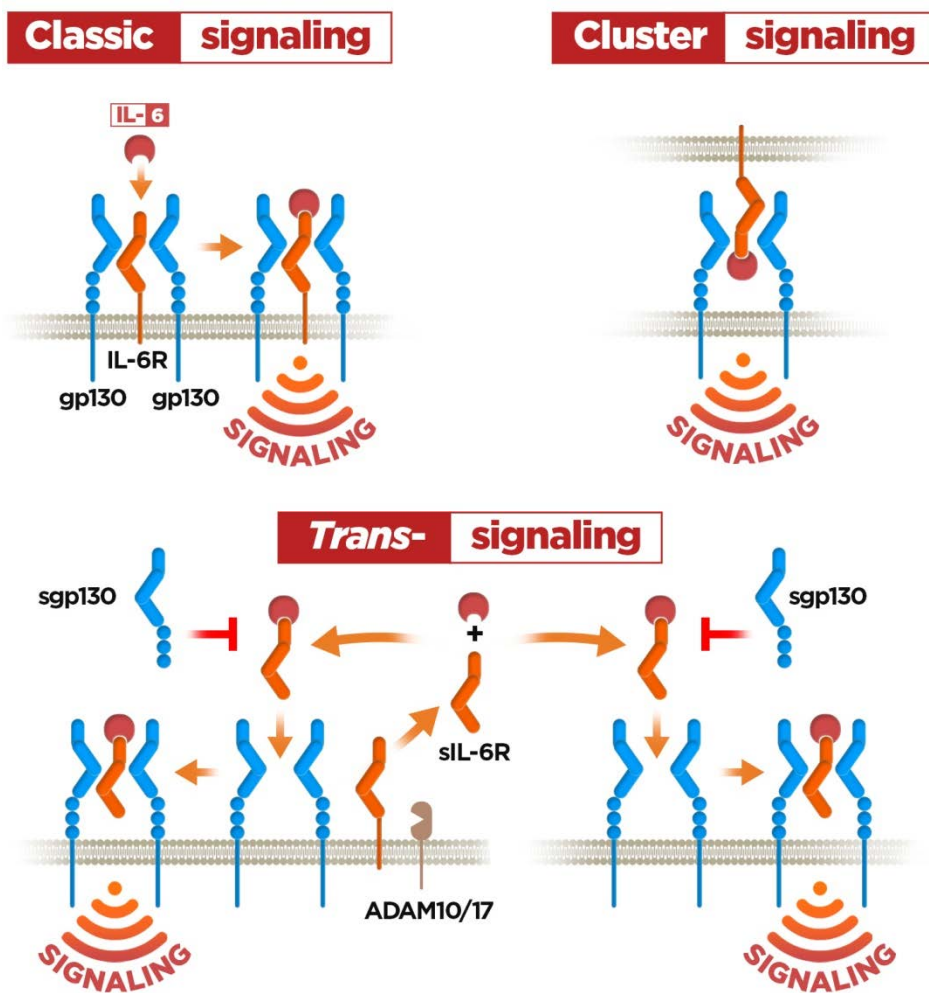

Figure 3. IL-6 signaling modes. Image adapted from (Scheller et al. 2011).

IL-6, IL-6R and gp130 expression

As stated previously, in the past, groups worldwide identified several proteins such as interferon-β2, B cell stimulatory factor 2 (BSF-2), hybridoma growth factor (HGF) or hepatocyte-stimulating factor (HSF) which after all, turned to be the same protein finally named IL-6. This fact highlights the diversity in expression and functions that this interleukin offers.

In vitro studies demonstrated that astrocytoma and glioma lines expressed IL-6 when stimulated with IL-1β (Yasukawa et al. 1987), demonstrating a role in the CNS. Therefore, both neurons (Marz et al. 1998) and glia (astrocytes, microglia, oligodendrocytes and Schawn cells) (Schöbitz et al. 1993; Yamabe et al. 1994; Bolin et al. 1995) express IL-6 in vivo to certain degrees throughout the brain. Besides, it has been demonstrated that endothelial cells of the irrigating blood vessels can also express IL-6 (Rott et al. 1993).

Besides, IL-6 is able to cross the blood-brain barrier (BBB) from the periphery by saturable transport to enter the cerebrospinal fluid (CSF) and brain parenchyma. However, it becomes rapidly degraded which limits the peripheral IL-6 contribution in the CNS. Even though, small amounts of intact IL-6 can induce CNS effects (Banks et al. 1994).

Outside the CNS, IL-6 is produced by a wide variety of different cells including monocytes, macrophages, fibroblasts, endothelial cells, keratinocytes, B cells and T cells and adipocytes (Heinrich et al. 1990).

As mentioned previously, gp130 is expressed ubiquitously, whereas the expression of IL-6R is limited to certain cell types. IL-6R is expressed in hepatocytes (Castell et al. 1988), megakaryocytes (Wolf et al. 2014) and some leukocytes, namely monocytes, macrophages, B cells and certain subtypes of T cells (Oberg et al. 2006). In the CNS, IL-6R protein is mostly detected in microglial cells (Hsu et al. 2015); therefore, IL-6 trans-signaling may play an important role inside the CNS.

IL-6 general functions

IL-6 is a pleiotropic cytokine which has been implicated in a wide variety of different processes. It has an important immunological function, indeed, IL-6 was firstly described as a B-cell differentiation factor (BSF-2) that stimulates the maturation of B

cells to antibody-producing cells (Hirano et al. 1985). Additionally, it is involved in the activation of acute phase response which is mediated by the innate immune system and provides a nonspecific mechanism to protect the body from infections and a variety of pathogens. In this response, acute phase proteins such as C reactive protein, fibrinogen or haptoglobin are secreted from the liver to fight pathogens (Heinrich et al. 1990).

IL-6 has the ability to orchestrate transition from innate to acquired immunity. Particularly, IL-6 trans-signaling has been involved in the chemokine-directed leukocyte recruitment to the site of infection and inflammation switching the balance from neutrophils to mononuclear leukocytes (Hurst et al. 2001); besides, it has also been implicated in the modulation of leukocytes apoptotic response (Jones 2005).

Furthermore, IL-6 could act as a growth factor. IL-6 enhances the proliferation of multipotential haematopoietic progenitors by synergizing with IL-3, IL-4, GM-CSF and M-CSF. Apart, it has been involved in growth and differentiation of other cell types. For instance, IL-6 stimulates human and murine leukaemia cells, induces morphological and functional differentiation into macrophages of certain types of cells and is involved in neuronal differentiation (Heinrich et al. 1990). Besides, IL-6 has been identified as a regulator of the pancreatic α-cells; it drives the α-cell mass expansion and glucagon expression during obesity (Ellingsgaard et al. 2008). Additionally, IL-6 has been implicated in angiogenesis (Gopinathan et al. 2015) and hepatic regeneration (Trautwein et al. 1996).

IL-6 is also an important regulator of bone homeostasis. Mice that overexpress IL-6 showed osteopenia (decreased osteoblast and increased osteoclast numbers) (De Benedetti et al. 2006). Besides, ovariectomized IL-6 deficient mice are protected of the loss of bone mass (Poli et al. 1994). This fact is relevant because its relation with menopause; as oestrogens suppress IL-6 production, in menopause, the oestrogens deficiency results in elevated IL-6 levels, bone loss and osteoporosis (Garbers et al. 2018).

Behavior

IL-6 is produced in several cell types in the CNS, thus, it is not surprising that it has been implicated in numerous normal behaviors. Effects on ambulation, exploration and anxiety have been attributed to this interleukin.

IL-6 KO mice have been employed in order to elucidate the normal role of IL-6 in behavior. Our group described increased emotional reactivity in novel environments in IL-6 KO mice generated from parental strains C57BL/6J and 129/SvJ (Kopf et al. 1994); these animals showed lower levels of ambulation in the HB and lower exploration of the open arms in the EPM suggesting increased anxiety (Armario et al. 1998) which is in agreement with other studies (Butterweck et al. 2003). Butterweck and colleagues also observed an increase in motility in OF, what seems in contrast with the results obtained by our group; however, reduced number of rearings were also observed, pinpointing the anxiolytic effect of IL-6 (Butterweck et al. 2003). When activity is measured in a familiar environment (i.e. home cages), the deficiency of systemic IL-6 affected in an age- and sex- dependent manner (Aniszewska et al. 2014). In contrast, other authors found no effect in ambulatory, exploratory and stereotypic activities in home cage or OF, multicompartment chamber and EPM using IL-6 KO mice with BALB.B background, evidencing the importance of the genetic background in behavioral assessment (Swiergiel and Dunn 2006).

The cell-specific deletion of IL-6 in transgenic mice also affected behavior. Astrocyte-IL6KO mice showed decreased ambulation in the HB and increased anxiety in the EPM in an age- and sex- dependent manner (Quintana et al. 2013; Erta et al. 2015) but consistent with previous results from the IL-6 KO mice (Armario et al. 1998). In contrast, in muscle-specific IL-6 KO male mice, increased ambulation and rearings were observed in the HB (Ferrer et al. 2014). These results highlight the importance of the source of IL-6 in its functions.

Regarding learning and memory, exogenous IL-6 suppresses long-term-potentiation (LTP) in hippocampus and impairs learning in rats (Li et al. 1997; Tancredi et al. 2000). Facilitatory effects were found in IL-6 KO mice in the radial maze (Braida et al. 2004). In line, GFAP-IL6 mice (overexpression of IL-6 under the GFAP promoter) displayed a decline in avoidance learning performance (Heyser et al. 1997). In contrast, other studies have found a detrimental effect in hippocampus- dependent and -independent learning in IL-6 KO mice measured using the Morris Water Maze (MWM) and in the Novel Object Recognition Test (NORT) (Baier et al. 2009), which is in accordance with the performance of astrocytes-IL-6 KO mice in MWM (Erta et al. 2015).

Besides influencing behavioral traits, IL-6 is also involved in sickness behavior. Sickness is a normal adaptive response to an infection, characterized by endocrine,

autonomic and behavioral changes such as malaise, decreased activity, diminished social interaction, decreased food and water intake and sleep dysregulation. The aim of this process is to reduce energy consumption and promote survival against the pathogen (Dantzer 2009). In fact, Burton and colleagues demonstrated that LPS-induced sickness behavior could be attenuated by the inhibition of the IL-6 trans-signaling in brain using an intracerebroventricular (ICV) injection of sgp130 (Burton et al. 2011).

Major depression and sickness behavior share symptoms and clinical signs. Indeed, in vulnerable individuals (by acquired or genetic factors), a sustained sickness behavior with sustained production of proinflammatory cytokines could lead to depression (Dantzer 2009). In humans, major depression is associated with elevated levels of circulating IL-6. Accordingly, studies in mice demonstrated that elevations in brain IL-6 levels contribute to depressive-like phenotype, with an important participation of trans-signaling (Sukoff Rizzo et al. 2012). In relation, IL-6 reciprocally participates in the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Bethin et al. 2000).

Energy metabolism

There are growing evidences identifying IL-6 as homeostatic regulator of energy and glucose metabolism being essential for the control of metabolism during health (Timper et al. 2017; Mishra et al. 2019). A single ICV injection of IL-6 increases energy expenditure (Wallenius et al. 2002b), in line, chronic ICV injection (Wallenius et al. 2002a) and adeno-associated viral delivery of IL-6 into hypothalamus (Li et al. 2002) decreases body weight and fat depots in rats.

Besides, total IL-6 KO mice displayed increased body weight, subcutaneous fat and dysregulation of glucose metabolism at advanced ages (Wallenius et al. 2002b; Matthews et al. 2010). Accordingly, GFAP-IL6 mice are resistant to high-fat diet-induced obesity (Hidalgo et al. 2010). However, other groups did not found the same weight changes in IL-6 KO mice (Di Gregorio et al. 2004) or with inhibited adipocyte-IL6 secretion (aP2-IL6 KO mice) (Navia et al. 2014). The deletion of IL-6 in muscle (mIL-6 KO mice) also influences body weight gain after a high-fat diet in a sex-dependent manner (Ferrer et al. 2014), which demonstrates again the importance of the source of IL-6 (Ferrer et al. 2014; Navia et al. 2014; Fernández-Gayol et al. 2019).

Apart from body-weight, different studies have observed effects of IL-6 on insulin signaling, as the acute IL-6 infusion impairs insulin action in mice (Kim et al. 2004) and the neutralization of IL-6 improve insulin resistance in inflammatory mouse models (Cai et al. 2005).

The CNS is suggested as the main site for the metabolic anorexic effects of IL-6 (Timper et al. 2017; Mishra et al. 2019), particularly, the hypothalamus is critical in the regulation of energy balance. The hypothalamus receives signals to balance energy expenditure and energy intake, thus, IL-6 together with energy balance signals like leptin (Timper et al. 2017) or glucagon-like-peptide-1 (GLP-1) (Shirazi et al. 2013), makes its effects on body weight regulation and metabolism. In fact, central application of IL-6 activates STAT3 signaling in hypothalamic neurons which suppresses feeding and improves peripheral glucose homeostasis (Timper et al. 2017). Apart from hypothalamus, the lateral parabrachial nucleus (lPBN) is also crucial in feeding control. IL-6 can be produced by lPBN neurons, astrocytes and microglia and acts in an autocrine and paracrine manner to reduce body weight by increasing brown adipose tissue (BAT) thermogenesis and by reducing food intake; these effects in the lPBN are executed in cooperation with leptin. Accordingly, the siRNA-mediated reduction of lPBN IL-6 and the observed reduction of IL-6 in the PBN from obese males mice lead to increased weight gain and adiposity, reduced BAT thermogenesis, and increased food intake (Mishra et al. 2019). In contrast, despite the changes in body weight, no changes in food intake were observed in GFAP-IL6 mice (Hidalgo et al. 2010) and adipose and muscular IL-6 KO mice (Ferrer et al. 2014; Navia et al. 2014) or total IL-6 KO mice in basal conditions (Wallenius et al. 2002b).

Obesity and IL-6

Obesity is frequently accompanied by a low-grade of inflammation and increased systemic IL-6 levels, mainly secreted by adipocytes (Mohamed-Ali et al. 1997; Roytblat et al. 2000); in fact, circulating levels of IL-6 correlate with adipose tissue mass (Mohamed-Ali et al. 1997; Vozarova et al. 2001). However, these changes do not correspond with IL-6 levels in the CNS, which seems to be reduced in this state (Stenlöf et al. 2003). These differences suggest that the function of peripheral and CNS IL-6 is divergent (Mishra et al. 2019).

Most of IL-6 metabolic effects in the CNS are done via trans-signaling (Timper et al. 2017). In line, neurons and astrocytes are almost not responsive to classical signaling

because of the lack in mIL-6R (Marz et al. 1998; Hsu et al. 2015). IL-6 trans-signaling has been involved in the recruitment of macrophages to adipose tissue in HFD-induced obesity. In the same study was observed that the inhibition of IL-6 trans-signaling in the periphery (using the sgp130Fc protein) in obese mice, unlike complete ablation of IL-6 signaling, prevented adipose tissue inflammation but without exacerbation of the HFD-induced insulin resistance or glucose intolerance. Thereby, the fact that sgp130Fc did not have adverse metabolic side effects suggests that it may represent a suitable therapeutic treatment for inflammatory diseases where IL-6 blockade is effective, avoiding the complete ablation of IL-6 signaling (Kraakman et al. 2015).

IL-6 and neuroinflammation

The CNS (brain and spinal cord) has been classically categorized as an immunologically "privileged" tissue; it was thought to be independent from the peripheral immune system, isolated by the BBB and immunologically inert. But, nowadays, the term immune privilege has changed its meaning, although the inflammatory response in the brain is different to that in the periphery. The CNS is immune competent and actively interactive with the peripheral immune system (Carson et al. 2003).

The term neuroinflammation refers to a brain-specific immune process comprising activation of the brain-resident cells, microglia and astrocytes. It is the first aid for the CNS against pathogens, being beneficial when it is acute and reversible. In contrast, it becomes damaging when persists too long and chronifies (DiSabato et al. 2016). In a pathological neuroinflammatory context, a wide range of different cytokines and chemokines are secreted, besides reactive oxygen species (ROS) and other inflammatory mediators (i.e. prostaglandins and acute phase proteins). Finally, in case of BBB disruption, the infiltration of peripheral immune cells is also produced (Heneka et al. 2015).

Astrogliosis

Astrocytes are the most abundant CNS-resident cells. They act as a neurosupportive cell, among its functions include secretion and recycling of transmitters, ion homeostasis, regulation of energy metabolism, synaptic remodeling, and modulation of oxidative stress (Wyss-coray and Rogers 2012). Reactive astrocytes are

characterized by hypertrophy, hyperplasia and a general upregulation of glial fibrillary acidic protein (GFAP) and vimentin (both cytoskeletal proteins involved in shaping the cell) (Sofroniew 2009). Numerous in vivo studies have demonstrated the link between the overexpression of IL-6 and increased astrogliosis; GFAP-IL6 mice (Campbell et al. 1993; Heyser et al. 1997) and NSE-IL6 (overexpression of IL-6 under the neuronal specific enolase (NSE) promoter) mice (Di Santo et al. 1996) showed increased astrogliosis. In accordance, a systemic overexpression in IL-6/IL-6R in transgenic mice (Brunello et al. 2000) or an infusion of IL-6 into a rat brain (Woiciechowsky et al. 2015) also led to an increment in astrocytes activation. In contrast, IL-6 KO mice displayed a reduction in astrogliosis after injury (Klein et al. 1997), besides, LPS-induced astrogliosis in rats can be inhibited by the intracerebroventricular (ICV) injection of an anti-IL-6 antibody (Pang et al. 2006). Furthermore, different studies in which downstream integrators from the IL-6 signaling pathway such as STAT3 were disrupted, showed a significant reduction of reactive astrocytes (Okada et al. 2006; Herrmann et al. 2008).

IL-6 affects astrocytic processes in many ways. It induces astrocytic proliferation (Selmaj et al. 1990; Guillemin et al. 1996) and chemotaxis by upregulating C-X-C motif 4 receptor (CXCR4) (Ödemis et al. 2002). It potentiates the proliferative effect of the endothelian growth factor (EGF) on aged astrocytes (Levison et al. 2000) and induces micro-RNA for cyclin-dependent kinase inhibitor 2A (CDKN2A), which elicits astrocytic proliferation (Pogue et al. 2010). IL-6 also increases the production of several inflammatory mediators by astrocytes such as chemokines, cytokines, prostaglandins and acute phase proteins (Kordula et al. 1998; Bolin et al. 2005; Quintana et al. 2008; Chikuma et al. 2009) as well as the expression of growth factors (März et al. 1999). Lastly, IL-6 drives astrogliogenesis (generation of new astrocytes) (Bonni et al. 1997; He et al. 2005).

Microgliosis

Microglia are tissue resident macrophages and comprise the 10-15 % of the total cells in the CNS (Cai et al. 2014). Microglia survey the brain for pathogens and support CNS homeostasis and plasticity (Heppner et al. 2015). Similar to astrocytes, the activation of microglia leads to changes in morphology, cell number and gene expression. Under resting conditions, microglial cells have a ramified morphology and lack of inflammatory properties, and when become activated they change to a more retracted and thicken morphology and start secreting cytokines and radical

species. Eventually, microglia become amoeboid cells capable of phagocytosis (Kreutzberg 1996; Ohsawa et al. 2004; Cai et al. 2014; Latta et al. 2015). Ionized calcium-binding adaptor protein-1 (IBA-1) is constitutively expressed in all microglia and became a usual immunohistochemical marker for both states (Ahmed et al. 2007). Apart from this morphological classification, a M1/M2 polarization has also been described differentiating between "classical activation", "alternative activation" and "acquired deactivation". M1 microglia courses with classical activation which is associated with the release of pro-inflammatory cytokines such as TNF-α, interleukin-1β (IL-1β), interleukin-6 (IL-6), superoxide, nitric oxide (NO) and ROS, whereas, M2 microglia includes both, alternative activation and acquired deactivation and it is associated with the release of anti-inflammatory cytokines as IL-4, IL-13, IL-10 or TNF-β (Orihuela et al. 2016; Tang and Le 2016).

The implication of IL-6 in microgliosis has been demonstrated with several animal models: IL-6 overexpression models display enhanced microgliosis in addition to astrogliosis, furthermore, IL-6 KO mice or treated with intracerebral anti-IL6 antibody also showed reduced microgliosis after nerve injury (Campbell et al. 1993; Di Santo et al. 1996; Heyser et al. 1997; Brunello et al. 2000; Pang et al. 2006).

Besides gliosis, one of the final stages in neuroinflammation is the disruption of the BBB and the extravasation of peripheral immune cells. IL-6 have an important role in the BBB maintenance influencing both, astrocytes and endothelial cells. IL-6 inhibits TNF-α-induced vascular cell adhesion molecule-1 (VCAM-1) upregulation (Oh et al. 1998) and TNF-α- or IL-1β- induced intracellular adhesion molecule-1 (ICAM-1) in primary astrocytes (Shrikant et al. 1995) acting as an anti-inflammatory cytokine and maintaining the BBB integrity in inflammatory conditions. In GFAP-IL6 mice a higher α5 and β4 integrin expression in astrocytes as well as higher laminin and fibronectin expression on their blood vessels were detected. Thus, during inflammation vascular cells accelerate their adhesive mechanisms in an attempt to increase stability of blood vessels (Milner and Campbell 2006).

Alzheimer's disease (AD)

Introduction

Alzheimer's disease (AD) was first described in 1906 when the German clinical psychiatrist and neuro-anatomist, Alois Alzheimer, reported "A peculiar severe disease process of the cerebral cortex". In November 1901, a female patient arrived to the Frankfurt Psychiatric Hospital. She was called August D. and she presented an untreatable paranoid symptomatology including sleep disorders, disturbances of memory, aggressiveness, crying and progressive confusion. When August D. died in 1906, Alzheimer was able to obtain brain material to describe the histological alterations later known as plaques and neurofibrillary tangles (Hippius and Neundörfer 2003).

Reflecting an aging population, AD is the most common diagnosed dementia in the industrialized world and it is becoming a major health burden for most societies (Ittner and Götz 2011). As Alois Alzheimer described briefly in 1906, clinically, AD is defined by a progressive loss of cognitive functions, memory and language; and neuropathologically by the aggregation of β-amyloid peptides (Aβ) forming extracellular "plaques" and hyperphosphorylated tau protein forming intracellular neurofibrillary "tangles" (NFTs). These changes are accompanied by neuronal loss, microvascular damage and pronounced inflammation in affected brain regions (Bertram et al. 2010).

AD is a multifactorial disease caused by many contributing factors, effects of multiple genes in combination with lifestyle and environmental agents. Although being a simplistic dichotomization, AD is usually divided in two forms: (1) familial cases with Mendelian inheritance and usually early-onset (<60 years people, [EOFAD]) and (2) sporadic cases with no genetic evidence and late-onset (>60 years people, [LOAD]) (Bertram et al. 2010).

(1) Early-onset familial Alzheimer's disease (EOFAD)

EOFAD represents approximately 5% of AD cases. It has a dominant Mendelian inheritance of highly penetrant mutations in mainly three genes: *APP*, *PSEN1* and *PSEN2* (Bertram and Tanzi 2004). *APP*, located in the chromosome 21q21, encodes for the Amyloid Precursor Protein, a transmembrane protein whose proteolysis

generates Aβ; *PSEN1* and *PSEN2*, located in chromosomes 14q24 and 1q31 respectively, encode Presenilin-1 and 2 proteins, which are part from the γ-secretase catalytic center. The three mutations are related to APP itself or its processing, leading to Aβ$_{40}$ and Aβ$_{42}$ overproduction and accumulation. Apart from these genes, potentially pathogenic mutations were reported in specific cases such as mutations in tau gene (*MAPT*) (Ostojic et al. 2004), PAX-interacting protein 1 gene (*PAXIP1*) (Rademakers et al. 2005) and in the Presenilin enhancer 2 gene (*PEN2*)(Frigerio et al. 2005). However, more information is needed to classify these mutations as risk factors for EOFAD (Bertram et al. 2010; Tanzi 2012).

(2) Late-onset Alzheimer's disease (LOAD)

LOAD sometimes is called "sporadic AD", however, 60-80% of this form is genetically determined. The combination of genetic and non-genetic factors makes difficult to find new loci consistently linked to the disease. Multiple reports suggest genetic association between AD and the common ε4-variant in the gene encoding apolipoprotein E (*APOE*) (Strittmatter et al. 1993; Bertram and Tanzi 2004), a lipoprotein involved in the metabolism of cholesterol (Michaelson 2014). Despite the association with AD, carrying the *APOE* ε4-variant it is not a determining factor to suffer the disease, it appears as susceptibility factor decreasing the age of onset, homozygous carriers show a younger onset age than hemizygous (Blacker et al. 1997). For many years *APOE* has been the sole candidate to LOAD, but now, other genes such as variants of the bridging integrator 1 (*BIN1* or amphiphysin2), *TREM2* and rare mutations in *ADAM10* gene postulate as strong candidates also (Kim et al. 2009; Tanzi 2012; Guerreiro et al. 2013; Tan et al. 2013). Some other genes have been related with LOAD, but the relationship is not yet clarified: *PLD3, ABCA7, CASS4, CD33, CD2AP, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB5-DBR1, INPP5D, MS4A, MEF2C, NME8, PICALM, PTK2B, SLC24H4 RIN3, SORL1, ZCWPW1* (Karch and Goate 2015).

Regarding non-genetic risk factors, aging is the most important for the sporadic AD form. Some age-related processes have been linked to Aβ aggregation as age-related neuronal stress, including oxidative stress (Arimon et al. 2015) and nitrosative stress (Guix et al. 2012), age-related inflammation and age-related disturbances in proteostasis (protein homeostasis machinery). Some diseases as type 2 diabetes and obesity has been also linked to AD with several proposed mechanisms such as insulin and insulin-like growth factor (IGF) resistance, glucose toxicity, oxidative stress and

mitochondrial dysfunction. Other AD-related factors are microbes of the human microbiome releasing large amounts of lipopolysaccharides (LPS) triggering the activation of pro-inflammatory cytokines, and moderate or severe traumatic brain injuries (TBI) (Zhang et al. 2018).
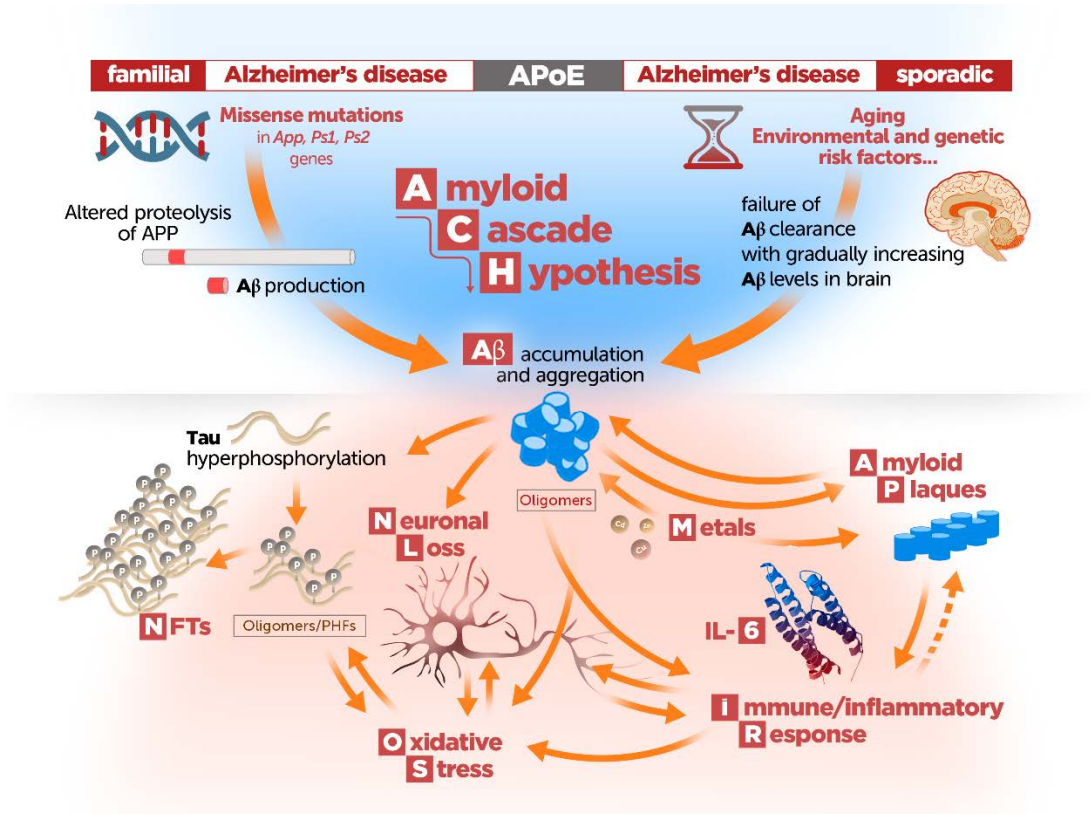
Figure 4. Schematic representation of AD risk factors and Amyloid Cascade Hypothesis. Adapted from (Comes 2017).

APP, β-amyloid (Aβ) plaques and Amyloid Cascade Hypothesis

As mentioned above β-amyloid plaques are one of the major hallmarks of AD. The plaque core consists in multimeric aggregates of Aβ, a 4 kDa polypeptide (Masters et al. 1985) cleaved from APP.

APP belongs to a family of evolutionary conserved and related proteins, that in mammals includes three different proteins: APP, APP-like protein-1 (APLP1) and

APP-like protein-2 (APLP2) (Wasco et al. 1993). Notably, only APP contains the sequence of the Aβ domain (Thinakaran and Koo 2008). Differential splicing of APP mRNA produces different variants of this protein; the major expressed isoforms have 770, 751 or 695 amino acid residues (Kang et al. 1987). APP_{695} variant is predominantly expressed in neurons, whereas APP_{770} and APP_{751} are more expressed in non-neuronal cells throughout the body (Dawkins and Small 2014).

The relationship between APP and AD has been fully studied but the physiological functions of APP remain unclear (Dawkins and Small 2014). The preserved sequence and the extensive expression and distribution suggest that APP plays important biological roles, including CNS development, synapse formation and function and brain neuroprotection following injury (Müller et al. 2017). More specifically, APP acts as a trophic factor; in vitro experiments have shown that sAPPα (proteolytic fragment of APP) alters the growth of different types of cells as fibroblast or neurons (Dawkins and Small 2014). Numerous studies demonstrated that APP is able to regulate the proliferation and differentiation of neural stem or progenitor cells (NSPCs) (Ohsawa et al. 1999; Demars et al. 2011; Baratchi et al. 2012). APP also regulates neurite outgrowth possibly managing cell-substrate (Clarris et al. 2002) or cell-cell adhesions (Soba et al. 2005). It also has a role in synaptogenesis being highly expressed in synapsis, especially during the critical period of synaptogenesis (Clarris et al. 1995). Furthermore, APP KO mice have a lot of deficiencies caused by a poor synaptic function (Ring et al. 2007). Additionally, APP is implicated in synaptic plasticity, learning and memory by affecting calcium signaling (Hoe et al. 2009). Lastly, APP has an anti-coagulant function since APP overexpressing mice showed a decreased cerebral thrombosis or severe haemorrhages, whereas, the opposite was observed in APP KO mice (Xu et al. 2005, 2007).

APP has been isolated, purified and sequenced from AD patients, familial Icelandic cerebrovascular amyloidosis syndrome patients and Down's syndrome patients (Glenner and Wong 1984; Masters et al. 1985). The latest present a third copy (or part) of the chromosome 21 and, hence, develop AD typical changes.

The increasingly advanced knowledge about APP has led to several AD hypotheses. In early 1990s, John A. Hardy and Gerald A. Higgins postulated the Amyloid Cascade Hypothesis. According to this hypothesis, the deposition of β-amyloid protein is the causative agent of AD pathology and the neurofibrillary tangles, cell loss, vascular damage, and dementia are direct results of this deposition (Hardy and Higgins 1992).

This hypothesis has evolved through the years, but the premise remains intact and it is still the most accepted hypothesis for AD. There are numerous evidences, mainly genetic, which support this hypothesis as some mutations in the *APP*, *PSEN1* and *PSEN2* genes (APP itself or genes involved in its processing) cause EOFAD (Goate et al. 1991). However, AD is associated with a complex biology and biochemistry, and there are growing amounts of data that are difficult to explain with a linear disease model. For instance, there are healthy subjects with large amounts of amyloid deposits who do not present AD symptoms. Similar results are obtained in transgenic mice with human *APP* constructs inserted in their genome; although they present signs of cognitive decline, never develop other typical AD signs as neurofibrillary tangles or neurodegeneration. Thus, the Amyloid Cascade Hypothesis seems to suit the familial cases, but it is still insufficient to explain the general AD pathology, especially the most prevalent sporadic form (Herrup 2015).

Apart from the Amyloid Cascade Hypothesis some others have been proposed, such as the cholinergic hypothesis based on a dysfunction in the choline acetyltransferase and acetylcholinesterase in AD patients, the tau hypothesis (Barage and Sonawane 2015) which proposes tau as the principal causative agent (Kametani and Hasegawa 2018) or the metal hypothesis of AD which propose that it is the interaction of Aβ with specific metals (copper and zinc) that drives Aβ pathogenicity. In this regard, brain's intrinsic supply of Cu^{2+} and Zn^{2+} (and possibly Fe^{3+}) mediates Aβ aggregation into oligomers and fibrils. Besides, Aβ induces oxidative stress by reducing these metal ions and producing H_2O_2, which is a substrate for the Fenton reaction that generates the highly reactive hydroxyl radical (OH•) (Bush and Tanzi 2008). Indeed, AD and oxidative stress are connected to each other since Aβ aggregation induces oxidative stress *in vivo* and *in vitro* and oxidative stress induces Aβ production by modulating or increasing expression and activity of Aβ producers enzymes (Guglielmotto et al. 2010; Arimon et al. 2015). In our lab, numerous studies have been carried out in order to elucidate the role of Metallothioneins (MTs) in AD, particularly in an AD mouse model (Tg2576). MTs are low molecular weight proteins with high metal content which in mammals include 4 isoforms (MT-1 to MT-4). Its isoform modulates AD phenotype in a sex and age-dependent manner, including changes in survival, behavior and Aβ content, suggesting an important role of these proteins and metals in this neurodegenerative disease (Manso et al. 2012a, b, 2016; Comes et al. 2017).

APP processing: from proteolysis to aggregation

APP is a type I transmembrane glycoprotein that is expressed in a wide variety of tissues including the nervous system, immune system, muscle, kidney, lung, pancreas, prostate gland and thyroid gland (Kang et al. 1987; Selkoe et al. 1988; De Silva et al. 1997).

APP is co-translationally translocated into the endoplasmatic reticulum (ER) and then, from the ER to the plasma membrane undergo through numerous post-translational modifications including glycosylation, sulphation, phosphorylation and palmitoylation. Most APP is incorporated to the endosomal-lysosomal system and, as a consequence, degraded. However, a portion (estimated ~ 10%) can be returned to the cell surface and being post-translationally processed by secretases. APP can initially be cleaved by α-secretase (non-amyloidogenic pathway) to produce and secrete sAPPα to the lumen/extracellular space and the membrane-associated C-terminal fragment CTFα or C83. Alternatively, APP can be cleaved by β-secretase (amyloidogenic pathway), which cuts 16-residues from the α-cleavage site, to produce the sAPPβ and CTFβ or C99 fragments (Thinakaran and Koo 2008; Dawkins and Small 2014). Responsible enzymes for α-secretase activity are members of the ADAM family including TACE/ADAM17, ADAM9, ADAM10 and MDC-9 and the aspartyl protease BACE2. The β-secretase has been identified as β-side APP-cleaving enzyme 1 (BACE1), it is a type 1 transmembrane aspartyl protease, homolog to BACE2 (Dawkins and Small 2014).

The resulting CTFs fragments can be further processed by γ-secretase to yield p3 or Aβ (depending on the pathway) to the extracellular space, and a short C-terminal peptide known as the APP intracellular domain (AICD). The γ-secretase is a transmembrane complex consisting of four protein subunits, presenilin-1 (PSEN1) or presenilin-2 (PSEN2), nicastrin, anterior pharynx-defective phenotype (APH-1) and presenilin enhancer 2 (PEN2). γ-secretase cleavage occurs in the transmembrane domain, starting from the C-terminal to the N-terminal domain at different cleavage sites termed γ-, ε- and ζ- generating Aβ peptides ranging from 39 to 42 residues, although $A\beta_{40}$ and $A\beta_{42}$ are the most prevalent forms (Dawkins and Small 2014). The γ-secretase cleavage sites have an important influence on the self-aggregating Aβ properties and the resulting pathogenicity, as only $A\beta_{42}$ peptide has a strong propensity to oligomerize in vivo (Haass and Selkoe 2007).
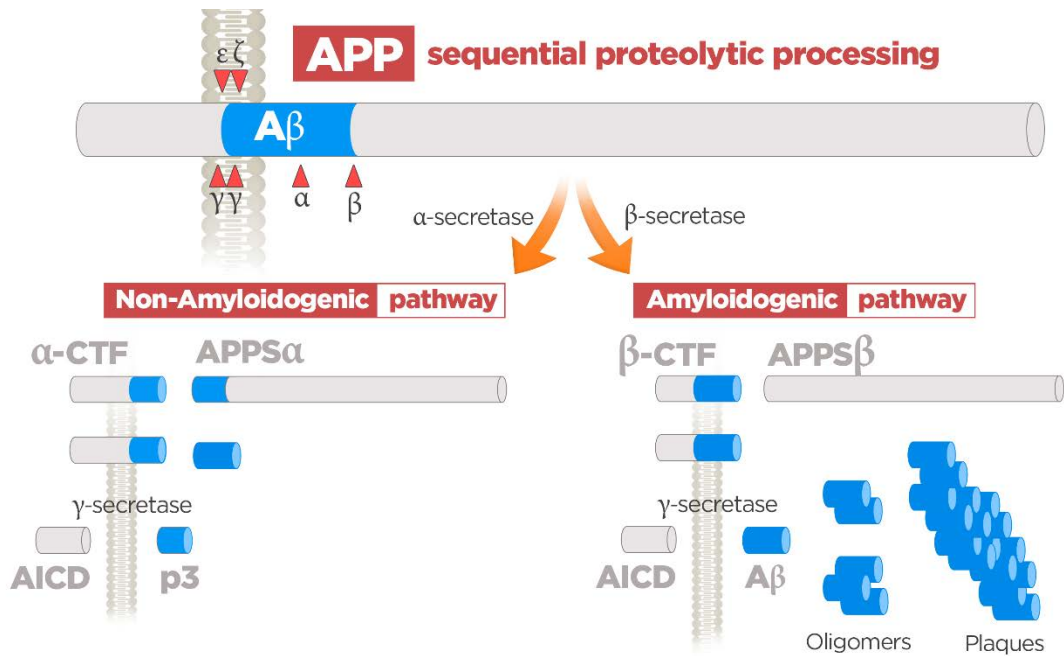
Figure 5. Schematic representation of APP sequential proteolytic processing. Aβ is generated through the amyloidogenic pathway. Aβ oligomerises and deposits into amyloid plaques. Adapted from (Comes 2017).

The amyloid plaques formation can be divided in three phases: (1) nucleation phase, (2) elongation phase and (3) stationary phase. In the nucleation phase, the precursors of aggregation (unfolded, intermediate and native proteins) assemble into oligomeric species. These oligomers can assemble to others forming, at the end, a fibril nucleus which can recruit more monomers and assembly into amyloid fibrils. During the elongation, amyloid fibrils are capable to elongate adding more prone species resulting in an exponential growth of fibrillar material. In the stationary phase, a dynamic balance between insoluble fibrils, oligomers and soluble monomers is achieved (Iadanza et al. 2018; Zhang et al. 2018).

A large number of different names have been given to different types of plaques with the objective to describe and classify them. Plaques could be classified as (Mrak 2009):

1. Diffuse, non-neuritic plaques: early, amorphous non-congophilic (not stained with Congo red) deposits of Aβ peptide without associated neuritic damage.

2. Diffuse neuritic plaques: later deposits in which some granular condensation of Aβ has occurred, but without a congophilic core, and in which there is some evidence of neuritic damage.

3. Dense core, neuritic plaques: central core of dense, congophilic amyloid is present.

4. Dense core, non-neuritic plaques: only the dense core persists, without associated diffuse amyloid and without associated neuritic damage.

Different types of plaques has been observed in all stages of the disease suggesting that each plaque evolves independently of the others (Dickson and Vickers 2001).

Aβ pathogenicity

As stated above, Aβ peptide has a central role in AD pathogenesis. The Amyloid Cascade Hypothesis postulates that the deposition of β-amyloid protein is the causative agent of AD pathology, but the feasibility of this hypothesis stills controversial, and the idea of Aβ oligomers being the primary impetus of the disease progression is taken advantage over β-amyloid plaques (De Strooper 2010). Modern laboratory techniques as specific Aβ enzyme-linked-immuno-sorbent assays (ELISAs), western blotting and mass spectrometry indicate that measured levels of soluble Aβ, including soluble oligomers, correlate better with the presence and degree of cognitive deficits than plaques amount. Although dissecting the role of fibrils, plaques and Aβ oligomers in AD symptoms is interesting it seems that, being a dynamic process, the combination of all species associates better with the disease (Iadanza et al. 2018).

The toxicity of oligomers and large fibril aggregates appears in the ability to disrupt essential cellular processes. The interaction of plaques with cellular membranes could interfere physically blocking the cellular exchanges or increasing the oxidative stress and inflammation, but not damaging intracellular organelles, although a study indicated that extracellular Aβ increases its intracellular concentration using an internalization mechanism by caveolae/lipid raft endocytosis (Saavedra et al. 2007). Both, extra and intracellular Aβ are able to induce apoptosis mediated via caspases proteins and the consequent neurodegeneration. This apoptotic process is created by early situations, including oxidative stress and inflammation. Additionally, some studies demonstrated the ability of soluble oligomers to disrupt membranes, exposing

cytoplasm to the extracellular space what causes calcium influx and ultimately cell death (Iadanza et al. 2018).

An imbalance between Aβ production and clearance is required to generate Aβ accumulation. A portion of Aβ is secreted to the bloodstream through receptors in the BBB, disruptions in these processes contribute to cerebral amyloid angiopathy (CAA), in which Aβ accumulates in blood vessels walls (Mackic et al. 1998; De Strooper 2010). The disturbances in the proteolytic machinery inside the brain also interfere in Aβ accumulation. Studies using transgenic animal models demonstrated that the overexpression of certain proteases can reduce Aβ levels or plaques, as well as, knockout mice showed the opposite. Some of the implicated Aβ degrading enzymes are neprilysin (NEP), endothelin converting enzymes 1 and 2, angiotensin converting enzyme and insulin degrading enzyme (IDE). Particularly IDE has higher affinity for insulin compared to Aβ, but hydrolyzes insulin at a much slower rate. Hence, insulin could act as an inhibitor of the IDE, providing then a possible link between type II diabetes, hyperinsulinemia and AD (De Strooper 2010; Murphy and LeVine III 2010).

Neurofibrillary tangles (NFT's)

Human tau is encoded by the *MAPT* gene, located on the chromosome 17. *MAPT* mRNA results in six tau protein isoforms generated by alternative splicing. The structure of tau is important for its normal functions; tau provides microtubule stability and contributes to the regulation of intracellular trafficking (Congdon and Sigurdsson 2018). It contains a low proportion of hydrophobic amino acids, thus, it is considered a hydrophilic protein. As APP, tau is subjected to post-translational modifications including phosphorylation, isomerization, glycation and nitration among others. Particularly, phosphorylation has big impact on its physiological function. Under pathological conditions the phosphorylation rate is increased (hyperphosphorylation); this fact reduces its affinity for microtubules, resulting in a cytoskeleton destabilization especially in neurons (Lindwall and Cole 1984). Lastly, the detached tau self-aggregate forming oligomers and heavier aggregates (Guo et al. 2017).

Tau deposition follows a stereotyped pattern; it begins in the entorhinal cortex and hippocampus before spreading to other regions (Braak and Braak 1997). In AD, tau exists as monomers, small oligomers and paired helical and straight filaments which

are the main components of the neurofibrillary tangles (NFTs) (Guo et al. 2017; Congdon and Sigurdsson 2018). Besides hyperphosphorylation, tau from AD patients is more acetylated than within brains of cognitively healthy individuals (Min et al. 2010). Additionally, it was demonstrated in tau isolated from AD patients that it undergone carboxy-terminal truncation by caspase 3. In AD, Aβ promotes caspase activation, providing then, a possible linkage between both hallmarks of the disease (Congdon and Sigurdsson 2018). Other modifications such as N-glycosylation is increased in AD and promotes phosphorylation and pathological conformational changes of tau (Wang et al. 1996), in contrast, O-GlcNAcylation (a type of O-glycosylation) seems to be protective against tauopathies (Liu et al. 2009).

Apart from the destabilization and self-aggregation there are alternative mechanisms for neurodegeneration. For instance, phosphorylation of tau can interfere in its intracellular route of degradation (Rodríguez-Martín et al. 2013), besides, when tau is hyperphosphorylated, the association to other interacting partners such as cytoplasmic membrane or DNA is altered, disrupting in consequence, several signaling pathways (Guo et al. 2017). Additionally, it has been demonstrated that the microinjection of tau into synaptic terminals increases calcium and disturbs synaptic transmission (Moreno et al. 2016).

The direct linkage between tau and Aβ is unclear, but GWAS studies demonstrated that APOE influences both. Additionally, studies with the 3xTg-AD, an AD animal model (which develops tangle and amyloid pathology), demonstrated that immunizing the transgenic animal with antibodies recognizing Aβ reduces phosphorylated tau (Oddo et al. 2004).

AD and inflammation

Inflammation has an important role in a large number of diseases including diabetes, multiple sclerosis, rheumatoid arthritis, obesity and also neurodegenerative diseases as AD. For instance, neuroinflammation contributes to AD pathogenesis and not in a passive manner. This is sustained by the increased amount of inflammatory cytokines, like TNF-α or IL-6, present in serum and brain of AD patients (Calsolaro and Edison 2016). Some evidences suggested that it have a causal role in the disease more than a consequence, as genes like *TREM2* or *CD33* have been associated with the disease. Thereby, immune processes may drive AD pathology from the beginning, exacerbating it and becoming a vicious pathophysiological cycle (Heppner et al. 2015).

Neuroinflammatory responses can be induced by both, CNS-intrinsic factors and systemic influences. Although, in AD the inflammatory response is especially driven (not entirely) by the CNS-resident immune cells as microglia and astrocytes (Heppner et al. 2015).

Microglia

The microglial reactivity is a complex multistage activation process. Indeed, while microglial activation could be beneficial at first aid, persistent inflammatory stimuli may overwhelm microglia and lead to chronic inflammation compromising the neuronal survival and contributing to the disease. It seems that in AD a mixed activation phenotype is showed. Interestingly, different forms of Aβ peptides may induce different inflammatory profiles, as the oligomeric Aβ induces M1 response stronger than the fibrillary form (Tang and Le 2016).

Once microglial response is activated, microglia cells migrate to the injury site to start the innate immune response. The detection of the threat is mediated via receptors that recognize danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs). Microglia is able to bind Aβ oligomers and fibrils and produce pro-inflammatory cytokines and chemokines to take part in Aβ clearance. Microglia can phagocyte Aβ fibrils which will be consequently degraded by the endolysosomal pathway. In contrast, soluble Aβ can be degraded by extracellular proteases. As stated before, inefficient clearance has been linked to sporadic AD cases (Heneka et al. 2015).

Recent studies using single-cell RNA sequencing identified a novel microglial type associated with neurodegenerative diseases (disease-associated microglia, DAM) which is spatially associated with sites of AD pathology. In addition, gene expression by DAM through disease progression revealed elevation of lipid metabolism pathways and phagocytic-related genes corresponding with the need for plaque clearance. This method allows to distinguish microglial actions from the other infiltrating immune cells; additionally, it allows the direct association between genes related to AD and microglia (Keren-Shaul et al. 2017).

Astroglia

Like microglia, they can be activated and respond to AD pathological stimuli. For instance, reactive astrocytes accumulate surrounding amyloid plaques and release inflammatory mediators such as interleukins or nitric oxide (Heppner et al. 2015).

Similar to microglia, astrocytes also have a beneficial role in Aβ clearance contributing to the microglial clearance capabilities through APOE and up-regulating the expression of Aβ-degrading proteases (Heneka et al. 2015).

Other CNS cells

Besides microglia and astrocytes other CNS-resident cells contribute to AD-related neuroinflammation such as oligodendrocytes, neurons, endothelial cells and other myeloid cells as perivascular macrophages. In fact, perivascular macrophages have a crucial role in the physiological removal of Aβ and protection from amyloid pathology (Heppner et al. 2015).

AD and IL-6

AD patients present an evident increase in IL-6 levels in brain and CSF (Bauer et al. 1991; Wood et al. 1993; Hüll et al. 1996). In fact, the rise in IL-6 occurs early in AD pathogenesis; hence, IL-6 has been proposed as a serum biomarker for AD (Bermejo et al. 2008).

Different outcomes has been discovered depending on the cellular source of IL-6 production, GFAP-IL6 mice displayed neurodegeneration and cognitive impairment (Campbell et al. 1993; Heyser et al. 1997), whereas, NSE-IL6 mice did not show effects on neuronal populations or behavioral deficits (Di Santo et al. 1996). Additionally, a number of studies demonstrated linkage between *IL-6* polymorphisms and AD. Although contradictory results have been found, one of the most investigated are −174 G/C bi-allelic polymorphism in the *IL-6* promoter and a variable number tandem repeat (VNTR) multi-allelic polymorphism in the *IL-6* 3′ untranslated region (Combarros et al. 2005; Koivisto et al. 2005; Ravaglia et al. 2006). Even so, as AD is a complex multifactorial disease, it is difficult to correlate specific *IL-6* polymorphisms with important changes in the pathology (Spooren et al. 2011).

IL-6 influences both hallmarks of AD, Aβ and tau. Aβ induces IL-6 expression in several brain cells (Chong 1997; Jana et al. 2008; Vukic et al. 2009) such as microglia via Toll-like receptors (TLR2) (Jana et al. 2008) or endothelial cells (Vukic et al. 2009).

At the same time, IL-6 induces the expression of APP in primary rat cortical neurons (Del Bo et al. 1995; Ringheim et al. 2002) and influences APP processing (Brugg et al. 1995). Besides, IL-6 enhances the neuronal damage induced by Aβ (Qiu and Gruol 2003). Regarding tau, IL-6 increases tau hyperphophorylation in primary rat hippocampal neurons (Quintanilla et al. 2004). All these evidences pointed out the detrimental effects of IL-6 in AD pathology. However, in vivo studies instigated opposite conclusions since the overexpression of IL-6 in APP transgenic mice leads to attenuated Aβ deposition and extensive gliosis at different ages activating the "beneficial" M2 microglia phenotype (Chakrabarty et al. 2010).

AD and IL-6 trans-signaling

Not a lot of investigations have been performed studying the relationship between IL-6 trans-signaling and AD. But, interestingly, IL-6 trans-signaling upregulates APP transcription in neuroblastoma cell lines (Ringheim et al. 2002). Furthermore, a polymorphism that increases total amount of sIL-6R may advance the age of onset of AD in APOE ε4 carriers (Haddick et al. 2017).

Trans-signaling is involved in the cellular communication of IL-6 in the CNS, particularly, it drives the pathogenic actions of IL-6 in the brain. Using a bigenic mice (termed GFAP-IL6/sgp130 mice) that coproduced IL-6 and the specific inhibitor of IL-6 trans-signaling (human sgp130Fc) in the brain, it was demonstrated that blocking the trans-signaling reduced many of the detrimental effects of IL-6 such as gliosis, vascular alterations, BBB leakage and neurogenesis impairment (Campbell et al. 2014).

Animal models of AD

Experimental models are necessary to understand AD pathogenesis, and also to develop possible therapies or testing potential drugs. Nowadays, the most used experimental models are the animal models. In general, the AD animal models can be divided in (1) spontaneous (without experimental manipulation) and (2) induced. There are some species including dogs, cats, polar bears, goats, sheep and several nonhuman primate which spontaneously develop amyloid plaques and, depending on the specie, tauopathies and cognitive decline. However, the use of these species is limited by availability, economical or ethical reasons. Regarding the induced animal models, they can be divided in (Van Dam and De Deyn 2011):

Pharmacological, lesion-induced and chemical rodent models

The majority are based on the cholinergic hypothesis of AD, since a correlation between cholinergic deficits, cognitive decline and neuropathological alterations has been reported in AD patients (Dournaud et al. 1995). Although with some limitations, the most used pharmacological model is the scopolamine-induced amnesia (Ebert and Kirch 1998), which antagonizes muscarinic receptors. In addition, blockade of nicotinic receptors also induce learning impairment (Riekkinen et al. 1990).

In the lesion-induced models, the lesion of the cholinergic centers can be achieved by different methods: surgical, electrolytical and intraparenchymal or intracerebroventricular microinjections of neurotoxic substances. This method allows better understanding about the role of cholinergic innervations in aethiology and treatment of the disorder. Furthermore, AD-related memory deficits can also be reproduced by lesioning brain structures related with these processes such as hippocampus, striatal and cortical regions. Finally, chemical induced models focus on mimic one specific pathophysiological pathway i.e. brain inflammation or glucose impairment (Van Dam and De Deyn 2011).

Amyloid-β infusion models

Aβ species can be administered acutely using a stereotaxic or repetitively through an implanted cannula. Direct injection causes learning and memory deficits, as well as, behavioral alterations. It also can lead to neuropathological changes as deficits in cholinergic function (Harkany et al. 1998; Yamada et al. 2005).

Transgenic models for AD

The majority consists in mice developing amyloid pathology or neurofibrillary tangles; however, other models as zebrafish or some invertebrates as *Drosophila melanogaster* and *Caenorhabditis elegans* have also been used. APP protein from wild-type mice has 97% sequence homology with human APP, but 3% difference includes the Aβ sequence, which prevents the formation of amyloid plaques. Therefore, the expression of human APP is necessary for the extracellular deposition of Aβ protein (Drummond and Wisniewski 2017).

Transgenic mice expressing human APP and PSEN1 with EOFAD mutations:

PDAPP mouse expresses human *APP* with the Indiana mutation (APP^{V717F}) resulting in plaque formation in cortex and hippocampus, gliosis and synaptic and cognitive impairment (Drummond and Wisniewski 2017). Tg2576 expresses human *APP* with the Swedish mutation ($APP^{K670N/M671L}$) under the control of the hamster prion protein (PrP) promoter. In the first paper characterizing this model, impairment in learning and memory appears at approximately 9 months of age, and it is correlative with the increment in Aβ amounts accompanied by amyloid plaques apparition in cortex, hippocampus and cerebellum (Hsiao et al. 1996). Since this paper, numerous studies have been performed using the Tg2576. Regarding behavioral characterization, increased explorative behavior and decreased anxiety was found, as well as, deficits in learning and memory even before the amyloid plaques apparition (Hsiao et al. 1996; Lalonde et al. 2003; Lassalle et al. 2008; Manso et al. 2012a, b, 2016). Additionally, increased astrogliosis and microgliosis is showed, predominantly surrounding plaques (Frautschy et al. 1998; Benzing et al. 1999). As well as, increased oxidative stress (Smith et al. 1998).

There are also models that combine multiple EOFAD mutations, as the J20 which combine Swedish and Indiana mutations or the APP/PS1 combining mutations in *APP* and *PSEN1*, which are faster in plaque formation than the previous ones. The most extreme is the 5xFAD which expresses the Swedish ($APP^{K670N/M671L}$), London (APP^{V717I}) and Florida (APP^{I716V}) APP mutations and the $PS1^{M146L}$ and $PS1^{L286V}$ mutations resulting in a very early intraneuronal Aβ accumulation at 6 weeks followed by plaque formation at 2 months (Drummond and Wisniewski 2017).

Transgenic mice expressing tau:

A wild-type mouse do no develop neurofibrillary tangles, besides, the expression of all six isoforms of human tau only results in tangles when the endogenous murine tau is inhibited. For these reasons, tau transgenic mice are built with human tau containing mutations associated with frontotemporal lobar degeneration (FTLD) developing, then, NFTs, neurodegeneration, atrophy and motor deficits. These mutations are not associated with AD in humans being a clear limitation of these transgenic mice (Drummond and Wisniewski 2017).

Transgenic mice with both plaques and tangles:

These models express together mutated forms of *APP*, *MAPT* and *PSEN1* or *PSEN2*. One of the more complete is the 3xTg-AD, where two independent transgenes

encoding $APP^{K670N/M671L}$ and Tau^{P301L}, under the Thy1.2 regulatory element as promoter, were comicroinjected in homozygous $PS1^{M146V}$ knock-in mice. These mice first develop intraneuronal Aβ at 3-4 months, followed by plaque formation at 6 months in cortex and hippocampus, whereas NFTs are formed at 12 months. Hence, Aβ pathology appears earlier than tau, which is consistent with the Amyloid Cascade Hypothesis. These mice present neurodegeneration, synaptic impairment and cognitive deficits accompanied by a prominent neuroinflammation. The majority of studies using this animal model has been performed in homozygous animals, but, hemizygous animals also present AD pathogenesis in a delayed manner (Oddo et al. 2003a, b).

Biomarkers and current treatments

A large number of investigations are being performed to understand AD pathogenesis and aetiology, many advances has been done in this regard, although no effective treatment is available.

Early detection is crucial for AD therapy; indeed, most therapies fail because the pathology is detected in the advanced stages. Thereby, it is important to identify reliable biomarkers to detect potential individuals in risk. Although $Aβ_{40}$, $Aβ_{42}$ and tau in CSF are accurate methods, blood-based biomarkers are more desirable, since the collection is less invasive and easily accepted by the patient. Despite the identification of potential blood-based biomarkers, no one has been validated to date (O'Bryant et al. 2017). Some studies showed that a ratio between parameters predict the progression of the disease with higher sensitivity and specificity than using a sole biomarker. Ratios as total-tau/$Aβ_{42}$, $Aβ_{42}$/$Aβ_{40}$ in association with Aβ positron emission tomography (PET) suggest that plasma biomarkers could be helpful for the differential diagnosis of AD. Apart from Aβ and tau, other promising biomarker is plasma neurofilament light (NFL)(Mattsson et al. 2017).

Imaging techniques provide an in vivo window to the pathological processes occurring in AD. Structural magnetic resonance imaging (MRI) is one of the most used; the scanners have a great ability to distinguish between grey and white matter and can detect subtle structural changes (Femminella et al. 2018). MRI studies have shown reduced hippocampal volume in AD patients, however, it is also detected in other neuropsychiatric disorders as schizophrenia (Steen et al. 2006), depression

(Arnone et al. 2012) or chronic stress (Mohlenhoff et al. 2014). Moreover, the evaluation of the BBB by MRI is being developed, although more studies are needed in this regard (Femminella et al. 2018).

The measurement of glucose consumption by fluorodeoxyglucose (FDG) PET is also a good method to detect AD with high predictive value and diagnostic power; glucose is an indicator of synaptic activity and a reduction in glucose metabolism is recognized as a biomarker for neurodegeneration. Brain amyloid imaging has become critical in AD diagnosis, in fact, PET-Aβ and tau have real impact on the medications and recommendations to patients and also, they are really useful in clinical trials. These methods help us to understand the cortical distribution of early pathological manifestations of AD. Additionally to these prototypical PETs, imaging astrocytes and microglia using PET is also possible (Femminella et al. 2018).

The majority of therapeutic strategies currently in the pipeline have Aβ as therapeutic target. They are focused in trying to reduce the production and/or increase the Aβ clearance, however, all of them still in clinical trials. BACE1 inhibitors have been tested, unfortunately, they fail in efficiency and did not pass the phase 3. Antagonists of 5-HT6 receptor have been proposed as cognitive enhancers and some 5-HT6-related drugs are being tested in different clinical trials. The same happen with tau-targeting therapies that are also being tested. Immunotherapy is also a possibility; however, the clinical trials are discouraging (Sun et al. 2018).

Current treatments for AD are more symptomatic than curative; they are focused on maintaining mental function, manage the behavioral symptoms and slow down memory loss without affecting the underlying neuropathology or its progression. In this regard, several medications has been approved by the U.S. Food and Drug Administration (FDA) to reduce AD symptoms. Donezepil (Aricept®), rivastigmine (Exelon®), and galantamine (Razadyne®) are cholinesterase inhibitors which partially restore the deficit in acetylcholine. Memantine (Namenda®) is a non-competitive modulator of the N-methyl-D-aspartate receptor and normalizes glutamatergic neurotransmission (National Institute on Aging 2016; Livingston et al. 2017). Additionally, systemic approaches are being studied since there are evidences that peripheral tissues and organs play important roles regulating tau and Aβ metabolism. The systemic strategy may provide a novel perspective for AD treatment (Sun et al. 2018).

A primary prevention can slow cognitive decline. One third of the dementia cases are preventable reducing risk factors as low early education, midlife hypertension and obesity, late-life depression, diabetes, hearing loss, physical inactivity, smoking and social isolation (Livingston et al. 2017).

Obesity and AD

As stated in the previous section, obesity and its metabolic or vascular comorbidities (i.e. insulin resistance, type II diabetes, hypertension) have been classified as a risk-factors for AD (Profenno et al. 2010; Alford et al. 2018). However, it is unclear if obesity has to be considered a direct causative agent or if it increases the risk through metabolic and vascular dysregulations.

Obesity courses with chronic systemic inflammation with increased cytokines secretion from the adipose tissue which achieve the brain from the periphery through the blood-brain barrier (BBB). Additionally, the liberation of cytokines from brain resident cells (microglia) has also been observed in this condition including IL-6 (Alford et al. 2018). Besides, insulin resistance, which is commonly present in obese patients, has an important role in AD pathogenesis. There are evidences that altered insulin actions facilitate Aβ and tau accumulation. Moreover, AD patients have attenuated insulin receptor expression in the hippocampus and hypothalamus (Talbot et al. 2012) and low ratio of insulin levels between the cerebrospinal fluid (CSF) and the plasma, suggesting a reduced insulin function in the CNS (Kleinridders et al. 2014; Alford et al. 2018).

IL-6 therapies

From the previous sections it is evident that IL-6 may be implicated in many pathologies, including AD and obesity. It is therefore not unreasonable to think that a treatment against IL-6 could help in these pathological situations. However, therapies against cytokines are complicated, whereas specific receptors for individual ligands exist, the majority of receptors are shared by several ligands. This fact is very important in designing therapeutic strategies. The direct blockade of a specific cytokine such as IL-6 only affects IL-6 downstream pathway. In contrast, if the target is a receptor such as IL-6R or gp130 the functions of other cytokines are also

compromised. Moving down the cascade, targeting a kinase or a transcriptor factor is the most unspecific way of intervention. However, many of these steps have been considered and many different inhibitors are being developed, or have even been evaluated in clinical trials. It is important to mention that IL-6 is required for the immune response against pathogens and, blocking its activity would compromise the efficacy of the immune system. In fact, the most prevalent side effects observed through the clinical trials targeting IL-6 or IL-6 receptors is the increased risk of bacterial infections (Garbers et al. 2018).

As stated before, a lot of therapeutic strategies are being designed targeting molecules through all the signaling cascade including IL-6 itself, IL-6R, IL-6/sIL-6R complex, gp130, JAK family and STAT3. However, not all these strategies are currently in the pipeline (see Table 1) since some of them still in basic investigation.

Generally, antibodies against IL-6 can block IL-6 classic signaling and IL-6 trans-signaling via the mIL-6R and the sIL-6R, respectively. Antibodies directed against IL-6R block all three types of IL-6 signaling (classic, trans-signaling and trans-presentation). Lastly, the blocking of the IL-6/sIL-6R complex, with the protein sgp130Fc, exclusively inhibits IL-6 trans-signaling without affecting classic IL-6 signaling or IL-6 trans-presentation (Garbers et al. 2018).

Table 1. Anti-IL-6 therapeutics in the pipeline. Extracted from (Garbers et al. 2018).

Name	Target	Company	Disease (clinical phase)
Sirukumab	IL-6	Centocor (Janssen)	Rheumatoid arthritis Depression Lupus nephritis
Olokizumab	IL-6	UCB	Rheumatoid arthritis Chron's disease
Clazakizumab	IL-6	Bristol Myers Squib and Alder	Organ transplant rejection
Siltuximab	IL-6	Janssen	Castleman disease Multiple myeloma
EBI-031	IL-6	Eleven Biotherapeutics and Roche	Diabetic macular oedema Uveitis
Tocilizumab	IL-6R	Chugai, Roche and Genentech	Rheumatoid arthritis Juvenile idiopathic arthritis Castleman disease Giant cell arteritis Cytokine release syndrome Neuromyelitis optica Systemic lupus erythematosus
Sarilumab	IL-6R	Sanofi and Regeneron	Rheumatoid arthritis
NI-1201	IL-6R	Novimmune and Tiziana	Not specified
Vobarilizumab	IL-6R	Ablynx	Rheumatoid arthritis Systemic lupus erythematosus
Olamkicept	IL-6 and sIL-6R	Conaris Research Institute Ferring Pharmaceuticals I-Mab Biopharma	Ulcerative colitis Inflammatory bowel disease
Tofacitinib	JAK3>JAK1>>JAK2	National Institutes of Health and Pfizer	Rheumatoid arthritis Chron's disease Psoriasis Ulcerative colitis
Ruxolitinib	JAK1 and JAK2	Novartis and Incyte	Myelofibrosis Polycythaemia vera
Filgotinib	JAK1	Galapagos and Gilead	Rheumatoid arthritis Chron's disease Ulcerative colitis
Baricitinib	JAK1 and JAK2	Eli Lilly and Incyte	Rheumatoid arthritis Psoriasis Systemic lupus erythematosus
Upadacitinib	JAK1	AbbVie	Ankylosing spondylitis Atopic dermatitis Juvenile rheumatoid arthritis Psoriatic arthritis Ulcerative colitis Rheumatoid arthritis Chron's disease
PF-04965842	JAK1	Pfizer	Atopic dermatitis

Sgp130Fc

To generate sgp130Fc, the cDNA coding the extracellular portion of human gp130 was fused to the constant region of a human IgG1 heavy chain. There is no species specificity; therefore it can inhibit human and murine IL-6/sIL-6R complexes. Furthermore, sgp130Fc turn out to be 10-100 times more efficient than sgp130, probably to the dimeric character of the Fc protein (Jostock et al. 2001; Campbell et al. 2014). All these features have made that it can be considered a suitable therapy for some inflammatory pathologies, so, as stated in the previous section, the sgp130Fc protein (Olamkicept) is in phase II clinical trial for the treatment of ulcerative colitis and inflammatory bowel disease (Garbers et al. 2018).
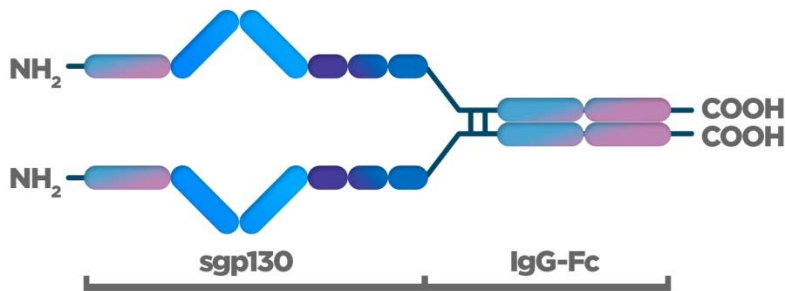

Figure 6. Schematic drawing of the soluble gp130 protein, sgp130Fc. Dark blue, fibronectin III domain; light blue, cytokine binding domain; blue-pink, Ig-like domain. Adapted from (Jostock et al. 2001).

To further study the role of IL-6 trans-signaling in IL-6 general functions and in pathology, two different animal models have been created (Rothaug et al. 2016). Firstly, a transgenic mice with sgp130Fc expressed under the control of a PEPCK promoter, which is liver specific; this mouse expresses the protein in the periphery, excluding the CNS (Rabe et al. 2008). Secondly, the GFAP-sgp130 with sgp130Fc expressed under the control of a GFAP promoter, which is astrocyte specific, has the protein expression restricted to the CNS (Campbell et al. 2014).

HYPOTHESIS AND OBJECTIVES

Due to the implication of neuroinflammation, and particularly IL-6 trans-signaling in AD, we hypothesize that the inhibition of IL-6 trans-signaling could modulate the phenotype of the Tg2576 and 3xTg-AD animal models. To this aim we propose the following objectives:

1. To obtain Tg2576 mice and 3xTg-AD mice with the IL-6 trans-signaling inhibited in the CNS and its negative controls.

2. To analyse physiological and behavioural parameters at different ages: survival, body weight, activity, exploration, anxiety and hippocampal-dependent learning and memory in both models.

3. To analyse the pathological hallmarks of the disease: APP processing, amyloid plaques and gliosis in both models.

4. To study how HFD-induced obesity affects AD hallmarks in the 3xTg-AD model.

5. To study how the inhibition of IL-6 trans-signaling modulates body weight and metabolism after HFD-induced obesity in the 3xTg-AD model.

CHAPTER 1

IL-6 trans-signaling in the brain influences the behavioral and physio-pathological phenotype of the Tg2576 mouse model of Alzheimer's disease.

Abstract

In this Chapter, we analyzed the role of IL-6 trans-signaling in a mouse model of AD, Tg2576 mice, which overexpress human Amyloid Precursor Protein (APP) with the Swedish mutation. The inhibition of IL-6 trans-signaling partially rescued the APP-induced mortality of females, and reversed APP-induced changes in exploration and anxiety before amyloid plaques deposition also in females, but not affected locomotion. The inhibition of IL-6 trans-signaling decreased amyloid plaque burden in cortex and hippocampus, whereas $A\beta_{40}$ and $A\beta_{42}$ levels were decreased in the former area only in the double transgenic females. However, the only behavioral trait affected was locomotion in a sex-dependent manner, reversing it in males and increasing it regardeless of APP expression in females. Spatial learning and memory were changed modestly by the blocking of the IL-6 trans-signaling. The aforementioned changes might be correlated with changes in blood vessels and matrix structure and organization rather than changes in neuroinflammation.

Material and methods

Generation of double transgenic Tg2576/GFAP-sgp130Fc mice

The parental strains used in this study, Tg2576 mice (Taconic Europe) and GFAP-sgp130Fc (provided by Dr. Stefan Rose-John), were generated and characterized previously (Hsiao et al. 1996; Campbell et al. 2014). To obtain the 4 genotypes of interest in the same genetic background, heterozygous Tg2576 (+/-) animals were mated with heterozygous GFAP-sgp130Fc (+/-). The resultant offspring was double transgenic Tg2576/GFAP-sgp130Fc (+/-)(+/-), Tg2576 (+/-)(-/-) mice, GFAP-sgp130Fc (-/-)(+/-) and WT (-/-)(-/-) mice. Genotype was determined by PCR analysis of tail DNA (see supplementary Material and Methods).

All mice used in this study were fed ad libitum and housed in a 12-hour dark-light cycle under specific pathogen-free conditions and constant temperature (22 ± 2ºC). All experimental procedures were approved by the Ethics Committee in Human and Animal experimentation (CEEAH 3971) from the Autonomous University of Barcelona and were in accordance with Spanish legislation and the EU directive (2010/63/UE) on 'Protection of Animals Used for Experimental and Other Scientific Purposes'. The study complies with the ARRIVE guidelines developed by the NC3Rs.

After weaning at 3 weeks of age, body weight and mortality were controlled regularly up to euthanasia. Animals were killed by decapitation to collect the brain. The left hemisphere was immersed in 4% paraformaldehyde (PFA) for 24 hours and stored in 70% ethanol at 4ºC until paraffin inclusion for immunohistochemistry (IHC). The right hemisphere was dissected into cortex and hippocampus and flash-frozen in liquid nitrogen for Western Blotting, ELISA and microarray analyses. All samples were stored at -80ºC until processed.

Behavioral tests

Mice were tested at ~4-6 (young, before amyloid plaques deposition) and at ~14-18 (old, after amyloid plaques deposition) months of age to characterize their behavioral phenotype, essentially, as described previously (Erta et al. 2015). All behavioral tests were carried out in the morning after half an hour of habituation to the room. One

test per day. The battery of tests were always preceded by five handling sessions in the testing room, where the animals were held during 10 seconds on the researcher's arm, and thereafter on the cage during 10 seconds more. All tests were carried out in a blind manner.

Figure 7. Battery of behavioral tests.

Open Field Test (OF)

Developed by Calvin S. Hall in 1934, it is a widely used model of anxiety-like behavior based on the exposition of the animal to an inescapable and bright but novel environment. It allows us to test exploration in a novel environment, general locomotor activity and an initial screen of anxiety-related behaviors in rodents (Walsh and Cummins 1976).

Mice were placed in the corner of a rectangular methacrylate white box (56 x 36.5 x 31 cm) and allowed to freely explore the apparatus for 5 minutes. The measured parameters were: total distance moved (horizontal activity), and defecations.

Hole-Board Test (HB)

The Hole Board apparatus was introduced by Boissier and Simon in 1962. They claimed that repeated head-dips reflect higher curiosity or desire to escape, more than basal activity because the animal is not used to the situation. Therefore, it is important

to distinguish between exploration and locomotor activity (Archer 1973; Brown and Nemes 2008) because they are no directly connected. The hole-board task is currently being used as a test of neophilia (Brown and Nemes 2008), interpreted by high levels of head-dipping, while, the lack of neophilia reflects an anxiogenic state in the animal (Takeda et al. 1998).

Mice were placed in the corner of an apparatus consisting of a square white methacrylate box (40 x 40 x 21.5 cm) with four equidistant holes and allowed to freely explore it for 5 minutes. The measured parameters were time and number of head-dipping (HD) events (considered to be when an animal inserted the head into the holes at least to its ear level).

Elevated-plus Maze Test (EPM)

Created by Pellow et al. in 1985, the Elevated-plus maze was firstly developed to test anxiolytic and anxiogenic drug effects in rats (Pellow et al. 1985). Nowadays, it is a widely used anxiety-related behavior assay for rodents. The test is based in rodent's tendency for darkness and closed areas upon the avoidance to light and open areas. Increased time and entries into light and open areas are related to anti-anxiety behavior (Walf and Frye 2007).

The EPM apparatus is a white elevated maze with four arms (20 x 5 cm), two open and two closed (15 cm high wall), connected by a central square (5 x 5 cm). The apparatus is elevated 50 cm from the floor. Mice were placed in the central square facing the closed arms and left to explore for 5 minutes. Entries and time in closed and open arms were measured. For analysis, the percentage of "time spent in" or "entries to" open arms was calculated (as the percentage from total time or entries to both open and closed arms, excluding the center).

Spatial learning and memory: Morris water maze (MWM)

Designed by Richard G.M. Morris in 1981 to test spatial learning in rats (Morris 1981). The MWM consisted of a propylene round pool (120 cm in diameter and 60 cm deep) virtually divided in four equal quadrants and a removable platform. It contains opaque (by adding white tempera) water at 22 ± 1°C and four 2D cues placed on the pool walls and four 3D cues placed before the black surrounding curtain. The following protocol was applied. The first day, the animals were exposed to a cued-learning session using a visible platform in order to check previous quadrant

preferences or visual deficits. From day 2 to 5, in the place task acquisition, mice had to escape from the pool by finding a hidden platform in a target quadrant (TQ) (submerged 1 cm below opaque water) using the cues as spatial references. Animals were allowed to explore the pool for 60 seconds and, in case they failed to find the platform, they were guided to it and required to remain on it for 15 seconds more. Four trials per day were performed and the escape latency (time required to localize the hidden platform) in each trial was recorded (~1 hour inter-trial). After this phase, short and long-term memory were tested in the probe trials administered ~1 hour (immediate) after the last training session (day 5) and 24 hours later (day 6), respectively. In the probe trials, the platform was removed and the time the mice spent in the TQ of the pool (the one where the hidden platform was located) out of a 30-second trial was measured. Additionally to this classic measurement, the latency to arrive to the position where the platform was located (± 5 cm) was analyzed.

From day 7 to 10, mice had to learn a new platform location in the reversal paradigm, when the location of the hidden platform was changed to the opposite quadrant. As before, the test was followed by two probe trial tests, immediately and 24 hours after (days 10 and 11).

Analysis of behavior

The data from the behavioral tests was obtained through both, in situ manual measurements (defecations and HD) and automated tracking analysis using specific software: ANY-Maze version 5.14 for the MWM test and EthoVision XT version 11.5 (Noldus) for the rest of them.

Neuropathological analysis

Mice ranging from approximately 16 to 19 month old were analyzed neuropathologically.

Western Blotting for Amyloid cascade (WB)

Brain samples were homogenized by sonication in a lysis buffer (50 nM TrisHCl, 150 nM NaCl, 2 mM EDTA, 0.1% Igepal CA 630 (Fluka), 3% SDS and 2% sodium deoxycolate) supplemented with a protease inhibitor cocktail and phenylmethylsulfonyl fluoride (Sigma-Aldrich).

Western blot was utilized to assess APP, Aβ, and other APP-derived proteolytic fragments such as CTF-β (6E10, Aβ1-16, 1:2,000, Biolegend). Monoclonal anti-β-Actin (1:5,000, Sigma-Aldrich) was used as loading control. Samples were loaded to a NuPAGE™ 4-12% Bis-Tris Midi Protein Gel (Invitrogen) and run for 70 minutes at 145 V (constant voltage). Proteins were transferred onto a 0.2 μm nitrocellulose membrane using the iBlot™ 2 Gel Transfer Device (Invitrogen). Membranes were blocked with 5% skimmed milk dissolved in TBS and incubated with the secondary antibodies (6E10 and Actin: Polyclonal rabbit anti-mouse Ig/HRP, 1:5,000, Dako). Immunoreactive bands were detected submerging the membranes 2 min in WesternBright™ ECL (Advansta) and imaging them in the Chemidoc Imaging System (Bio-Rad). Images were analysed using Image Lab™ Software (Bio-Rad).

Enzyme-Linked ImmunoSorbent Assays (ELISAs)

ELISA kits were used to measure $A\beta_{40}$, $A\beta_{42}$ and sgp130Fc levels in brain samples (Amyloid beta 40 and 42 Human ELISA Kits, ThermoFisher; Human sgp130 DuoSet ELISA, R&D systems). Brain samples were homogenized as in WB section.

Immunohistochemistry (IHC)

Left hemispheres were paraffin-embedded and cut sagitally in 8 μm thick sections to detect Aβ plaque load (4G8, 1:5,000, Signet), astrogliosis (GFAP, polyclonal rabbit anti-GFAP, 1:900, Dako), and microgliosis (Iba1, polyclonal rabbit anti-Iba1, 1:1,500, Wako) in cortex and hippocampus. Briefly, slides were deparaffinised and washed 3 times with TBS (Tris-buffered saline, 0.05 M, pH = 7.4), incubated for 15 minutes in 70% methanol and 2% H_2O_2 for the peroxidase inhibition, washed again 3 times TBS-T (TBS-Triton 0.5%, 0.05 M, pH = 7.4) and blocked during 1 h with 0.5% BSA (Bovine Serum Albumin, Sigma) in TBS-T. A pre-treatment with Citrate Buffer (10mM, 0.05% Tween, pH = 6, 20 minutes) and Formic acid (70 % in TBS, 20 minutes) was required for Iba1 and 4G8, respectively. Slides were incubated o/n at 4 °C with the primary antibody. Slides were washed with TBS-T and incubated with the secondary antibody (4G8: biotinylated anti-rabbit IgG 1:400, SIGMA, B-9904; GFAP and Iba1: biotinylated anti-rabbit IgG 1:300, Vector Laboratories, BA-1000) for an hour and with Horseradish Streptavidin Peroxidase for also 1 h. 3,3'-Diaminobenzidine (DAB) was used as a chromogen. Finally, slides were washed with TB (Tris-buffered, 0.05 M, pH = 7.4), dehydrated and mounted with DPX mounting medium.

For quantification, two non-consecutive sections per mouse were used. Two measures were taken in each region, the area occupied by specific signal and the intensity (volume) of the specific signal (integrated density, staining quantity). Serial pictures of cortex and hippocampus were taken with a Nikon Eclipse 90i microscope (2X and 10X). Images were analyzed using ImageJ software.

Microarray

An oligonucleotide array analysis was performed in hippocampus of 16 to 19-month old female mice (n=4 per group) by Bioarray S.A. Quality of RNA samples was determined using the R6K ScreenTape kit (Agilent). A SurePrint G3 Mouse Gene Expression v2 8x60K Microarray (ID 074809, Agilent) was used, followed by the Two-Color Microarray-Based Gene Expression Analysis v. 6.5. protocol (Agilent).

For statistical analysis a Two-way ANOVA was applied with APP and sgp130Fc as main factors to detect differentially expressed (DE) genes (p-value < 0.05). The DE candidate genes were additionally filtered for a fold change of at least 1.5 in any of the pairwise comparison of interest (WT vs. GFAP-sgp130, WT vs. Tg2576, GFAP-sgp130 vs. Tg2576/GFAP-sgp130, Tg2576 vs. Tg2576/GFAP-sgp130). Heatmaps were generated as graphical representation of the DE genes (Metsalu and Vilo 2015).

Finally, gene ontology (GO) (Eden et al. 2009) enrichments of the DE genes were carried out to identify biological categories overrepresented in these regulated genes.

Statistics

Statistical calculations were done using the Statistical Package for Social Sciences (SPSS, version 19). Males and females were analyzed separately. Most data were analyzed using the Generalized Linear Model (GLZ) with APP (APP positive vs. APP negative) and sgp130Fc (sgp130Fc positive vs. sgp130Fc negative) as main factors. Generalized estimated equations (GEE) were used for repeated measures (i.e., body weight, escape latencies in MWM) including also both genotypes as main factors and time as a within-subject factor. In case of comparisons between two groups, the t-Student was used. Survival was analyzed using the Kaplan–Meier and Log rank survival tests, using genotype as a factor with four levels (WT, GFAP-sgp130Fc, Tg2576, Tg2576/GFAP-sgp130Fc). Statistical significance was defined as $p \leq 0.05$.

In the analysis of amyloid plaques deposition by IHC, an outlier animal, which expressed high amount of 4G8 signal, was excluded from the double transgenic female group and it was also excluded in the posterior analysis of amyloid pathology, including Aβ ELISAS and amyloid cascade western blots.

Results

The inhibition of IL-6 trans-signaling rescued APP-induced premature mortality, but not the diminished body weight, in a sex-dependent manner.

Analysis of the Mendelian distribution of the mouse litters at weaning revealed a decreased intrauterine and/or perinatal survival associated with the presence of the *App* transgene in females, but no significant effect of the presence of sgp130Fc was observed. On the other hand, no effects of either APP or sgp130Fc were observed in males (Fig. 8A).

Additionally, a clear trend in APP positive mice to increased mortality was observed in both sexes, significantly in females, reaching approximately 50% at advanced ages. The inhibition of the IL-6 trans-signaling partially rescued this decreased survival in females; the same trend was observed in males, but it was not significant (Fig. 8B).

APP positive animals also showed lower body weight in comparison with the control animals in both sexes. However, the presence of sgp130Fc protein did not affect this parameter (Fig. 8C).

The presence of sgp130Fc in brain was evaluated using a specific ELISA, no differences between sexes were observed in total amount of protein (Fig. 8D).

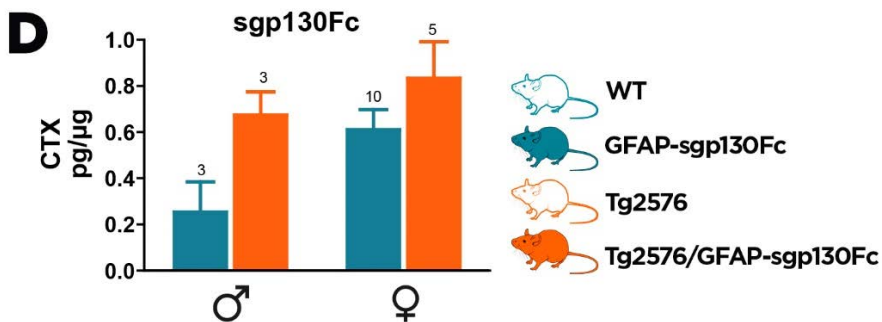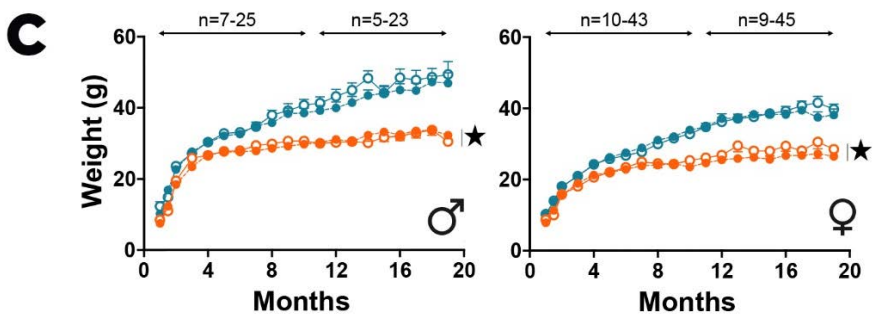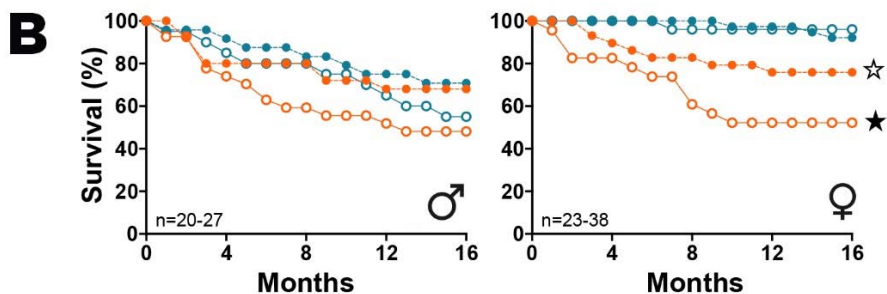| Births		APP+	APP-	Total Sgp130Fc
Sgp130Fc+	♂	35 ⎤ 64	35 ⎤ 87	70 ⎤ 151
	♀	29 ⎦	52 ⎦	81 ⎦
Sgp130Fc-	♂	38 ⎤ 63	26 ⎤ 65	64 ⎤ 128
	♀	25 ⎦	39 ⎦	64 ⎦
Total APP	♂	73 ⎤ 127	61 ⎤ 152	279
	♀	★ 54 ⎦	91 ⎦	

Figure 8. Effect of the inhibition of IL-6 trans-signaling in life span and body weight. (A) Mendelian distributions at weaning indicated an increase in females' mortality associated with the *App* transgene (observed: 54, expected: 72.5, χ2 = 9.441, p<0.002 with one degree of freedom). No effect of the presence of sgp130Fc protein was observed. As well as, no differences were observed in males. (B) In females, sgp130Fc partially reversed the detrimental effect of the *App* transgene in survival. No effects were observed in males, although a trend is showed. (C) APP positive animals showed reduced body weight compared to APP negative animals. (D) Sgp130Fc was detected in cortex from sgp130Fc positive males and females; no differences between sexes were observed in total amount of protein. Results are MEAN ± SEM. ★ *p ≤ 0.05 vs. APP negative;* ☆ p ≤ 0.05 vs. WT.

The presence of sgp130Fc modulates modestly and only in females some APP-dependent behavioral traits before amyloid plaques deposition.

Mice from both sexes were characterized behaviorally before the amyloid plaques deposition, at 4-6 months of age. Using the OF, no differences in locomotion were detected in males, but, APP positive females showed an increased mobility. The presence of sgp130Fc did not affect activity. An increase in defecations was observed in APP positive males, whereas no differences were observed in APP positive females. No effects of the inhibition of IL-6 trans-signaling were observed in this case (Fig. 9A left).

Regarding exploratory behavior in the HB, *App* transgene expression increased exploration in females. The inhibition of the IL-6 trans-signaling reversed this effect when time of head-dipping was measured. No differences were observed in males (Fig. 9A middle).

Anxiety was evaluated with the elevated-plus maze (EPM). APP positive animals were less anxious than control mice being the effect more prominent in females. This effect was partially reversed by the presence of sgp130Fc protein in females (Fig. 9A right).

Spatial learning and memory was analyzed using the Morris Water Maze test. During the acquisition phase, APP positive males showed an impaired learning as they needed more time to find the submerged platform. They also showed impaired retention during the immediate and 24h probe trial tests. In females, no significant learning deficits were observed, but, a deficit in short and long-term spatial memory was detected in both probe trials. No effects of the inhibition of IL-6 trans-signaling were

observed in this test phase (Fig. 9B top). When the animals were challenged to find a new platform location, APP positive females showed learning deficits, more pronounced in the Tg2576 group. In the subsequent probe trials, APP positive females showed retention deficits 24h after the last training session; the inhibition of IL-6 trans-signaling decreased the time spent in the TQ in the immediate probe trial. No differences between groups were observed in males (Fig. 9B bottom).
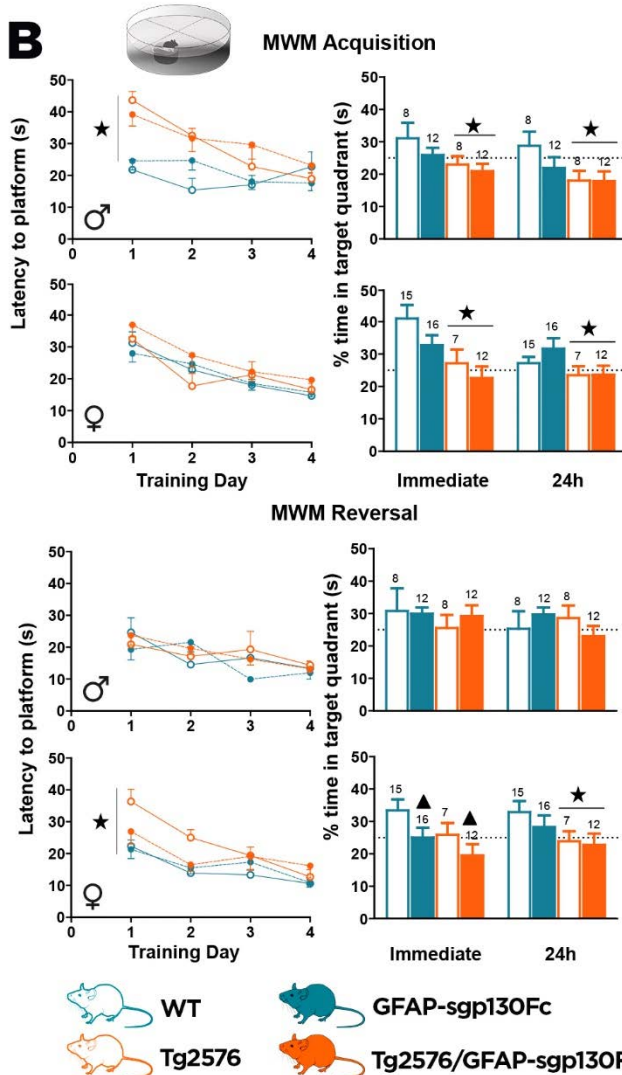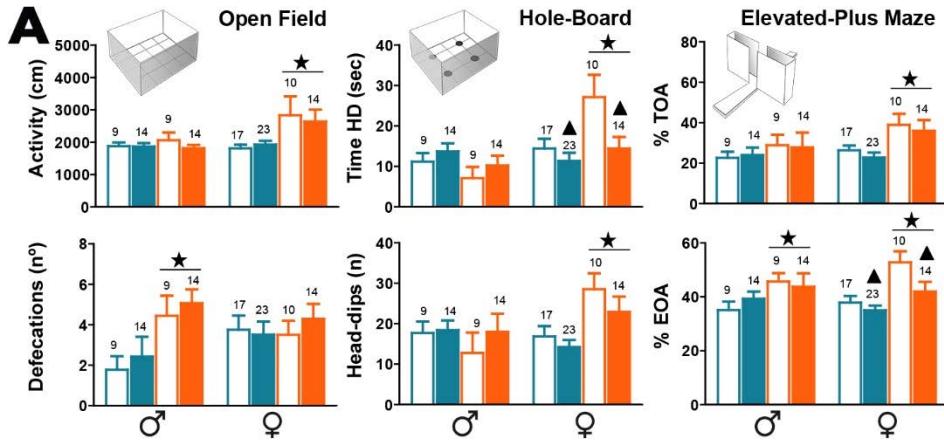
Figure 9. Effect of the inhibition of IL-6 trans-signaling on behavior before amyloid plaques deposition. (A) APP positive females displayed increased activity (OF) and exploratory behavior (HB), as well as, decreased anxiety (EPM) which was also observed in males. The presence of sgp130Fc reversed partially the effects on exploration and anxiety, only in females. In addition, APP positive males presented increased defecation compared to APP negative animals. (B, top) In the MWM, APP positive animals showed deficits in retention during the probe trials from the acquisition, besides, APP positive males exhibited also higher latencies to find the platform during the respective training days. (B, bottom) In the reversal test, APP positive females presented deficits in learning and memory showed by increased latencies to find the platform during the reversal training days and reduced time spent in the TQ in the 24h probe trial; sgp130Fc reduced even more retention in females, 1h after the last training trial. Results are MEAN ± SEM. ★ *p≤ 0.05 vs. APP negative;* ▲ *p≤ 0.05 vs. sgp130Fc negative.*

The inhibition of IL-6 trans-signaling affected locomotion measured by OF in a sex-dependent manner after the amyloid plaques deposition.

The same parameters than in young animals were assessed after β-amyloid deposition in mice ranging from approximately 15 to 18 month-old. APP positive mice from both sexes presented an increase in locomotion in the OF; the inhibition of IL-6 trans-signaling reversed this effect in males, whereas, in females, it increased activity independently from *App* transgene (Fig. 10A left).

In the HB, an increment in exploration was observed in APP positive females in comparison with the negative littermates. The presence of sgp130Fc did not affect this trait at this age (Fig. 10A middle).

APP positive animals were significantly less anxious than APP negative, as evidenced by their performance in the EPM. No effects of the IL-6 trans-signaling inhibition were also observed at this age (Fig. 10A right).

In general, behavioral traits were attenuated in comparison to younger ages, presumably as a consequence of the reduced mobility of aged mice.

The MWM test was performed only by females. When the animals had to locate the submerged platform following the external cues APP positive animals showed impaired learning and memory. The learning deficits were in accordance with the poor retention showed during the following probe trials by the APP positive genotypes, with no effect of the IL-6 trans-signaling inhibition when the time spent in

the target quadrant is analyzed. In the reversal test, the same deficits in learning and memory as in the acquisition test were detected. In the probe trials, APP positive females showed retention deficits 24 hour after the last training session; no effects of the IL-6 trans-signaling were observed at this point. In contrast, immediately after the last training session, the presence of sgp130Fc protein diminished the time spent in the TQ independently from *App* transgene (Fig. 10B).
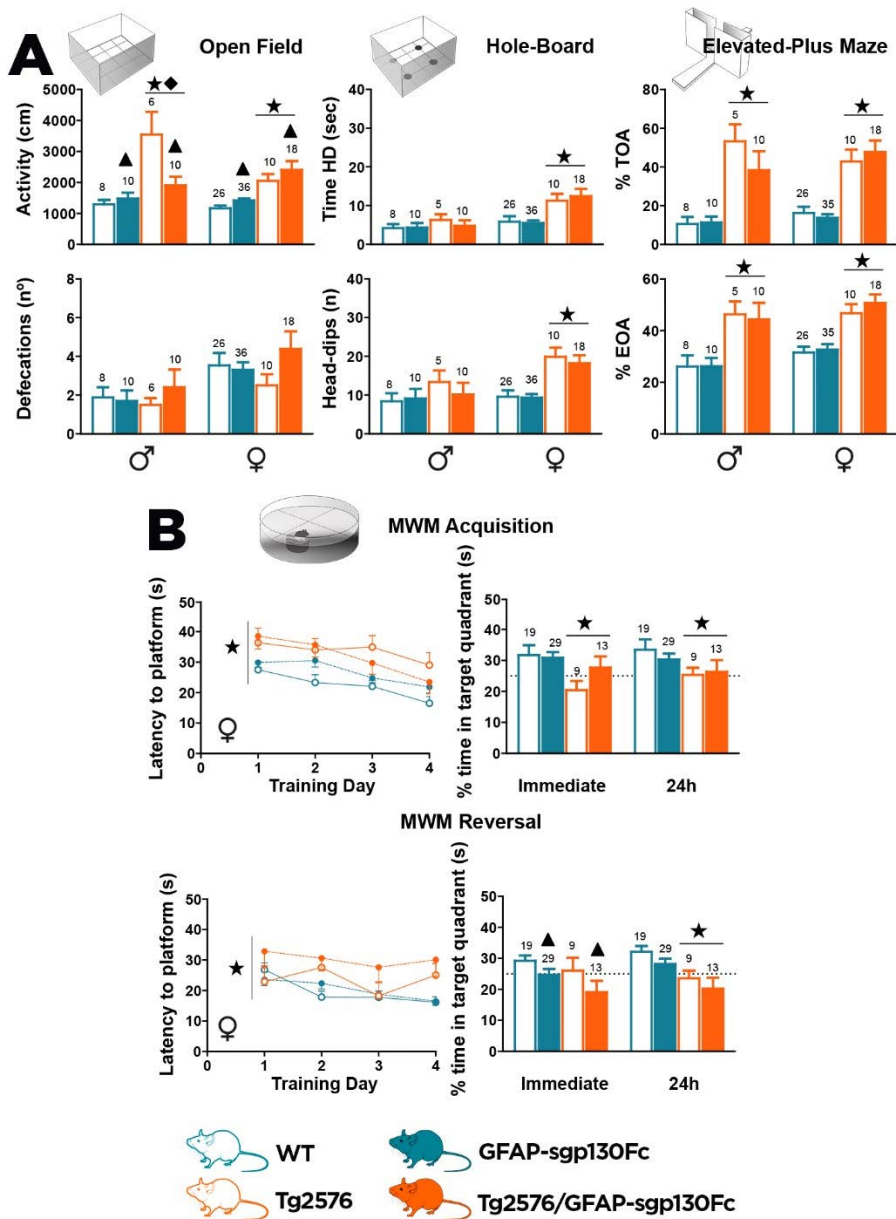
Figure 10. Effect of the inhibition of IL-6 trans-signaling on behavior after amyloid plaques deposition. (A) In the OF, APP positive animals showed increased activity; the inhibition of the IL-6 trans-signaling had sex-dependent effects in this parameter. In the HB, APP positive females displayed an increased exploratory behavior, with no effects of the sgp130Fc protein. Lastly, APP positive animals showed reduced anxiety, again, not affected by the presence of sgp130Fc protein. (B) Using the MWM, deficits in learning and memory were observed in females in both test phases; sgp130Fc protein had a detrimental effect on retention in the reversal immediate probe trial. Results are MEAN ± SEM. ★ $p \leq$ *0.05 vs. APP negative;* ▲ $p \leq$ *0.05 vs. sgp130Fc negative.*

The inhibition of IL-6 trans-signaling reversed the amyloid plaques deposition in females, with no effect on gliosis.

The amyloid plaque load was assessed in cortex and hippocampus from male and female mice by IHC. The age of the mice ranged from approximately 16 to 19 month old. As expected plaques were only detected in APP positive mice in both brain areas. Although no effect of the presence of sgp130Fc was observed in males, in females, the inhibition of IL-6 trans-signaling decreased plaque load in both regions (Fig. 11B). It is worthy to mention that both sexes were analyzed in different batches; therefore, the total amount of 4G8 staining is not comparable between them.

Figure 11. Effect of the inhibition of IL-6 trans-signaling on amyloid load in cortex and hippocampus from ~16-19 month-old mice. (A) Representative immunostaining for amyloid plaques. (B) Quantification of Aβ immunostaining in cortex (continuous line) and hippocampus (dashed line). The presence of sgp130Fc reduced the amyloid plaque deposition in both areas from APP positive females. Results are MEAN ± SEM. ▲ *p≤ 0.05 vs. sgp130Fc negative.*

Usually amyloid plaques are accompanied by gliosis, GFAP (Fig. 12B) and IBA-1 (Fig. 12D) markers were analyzed by IHC in both regions. In females, an increase in astrogliosis and microgliosis was observed in APP positive mice in both structures. In contrast, only APP positive male's cortexes showed an increment in astrogliosis, whereas it was not detected in hippocampus or Iba-1 staining. The inhibition of the IL-6 trans-signaling did not have an effect on these inflammatory markers.
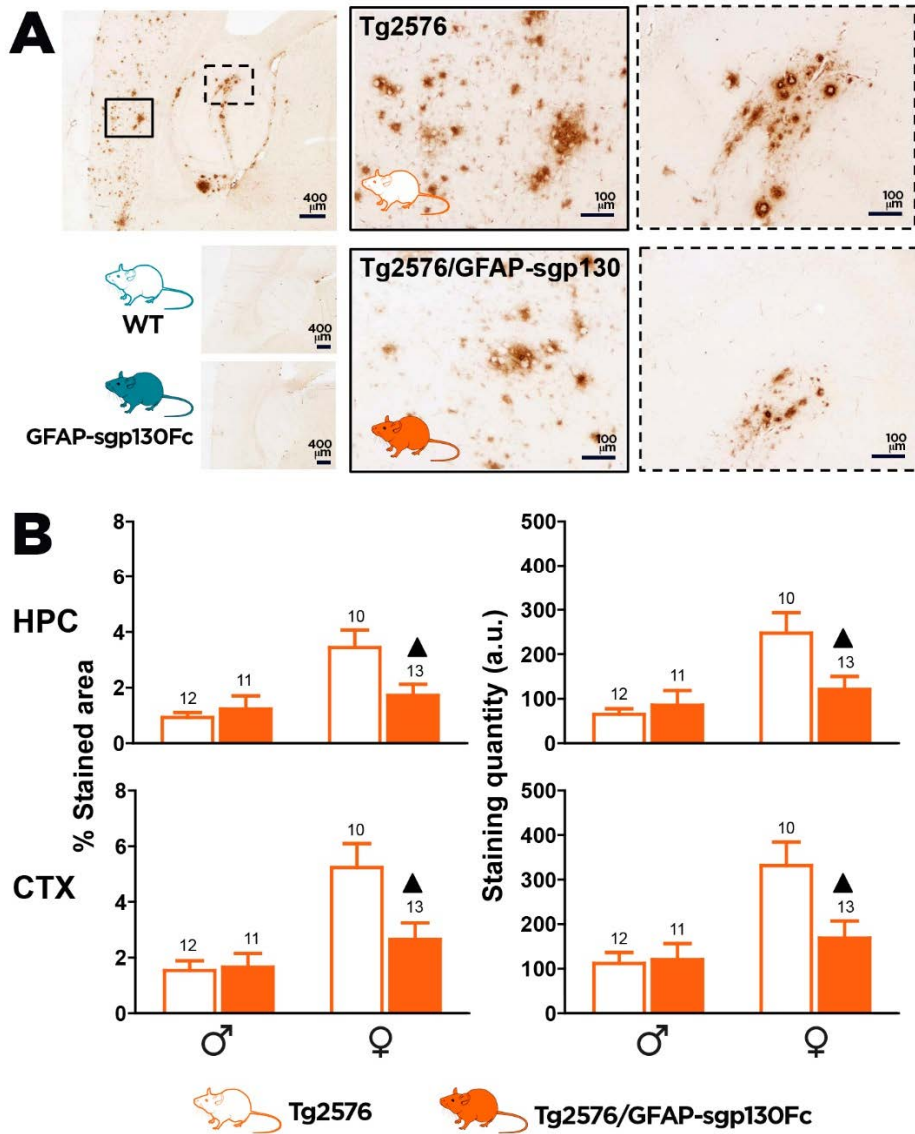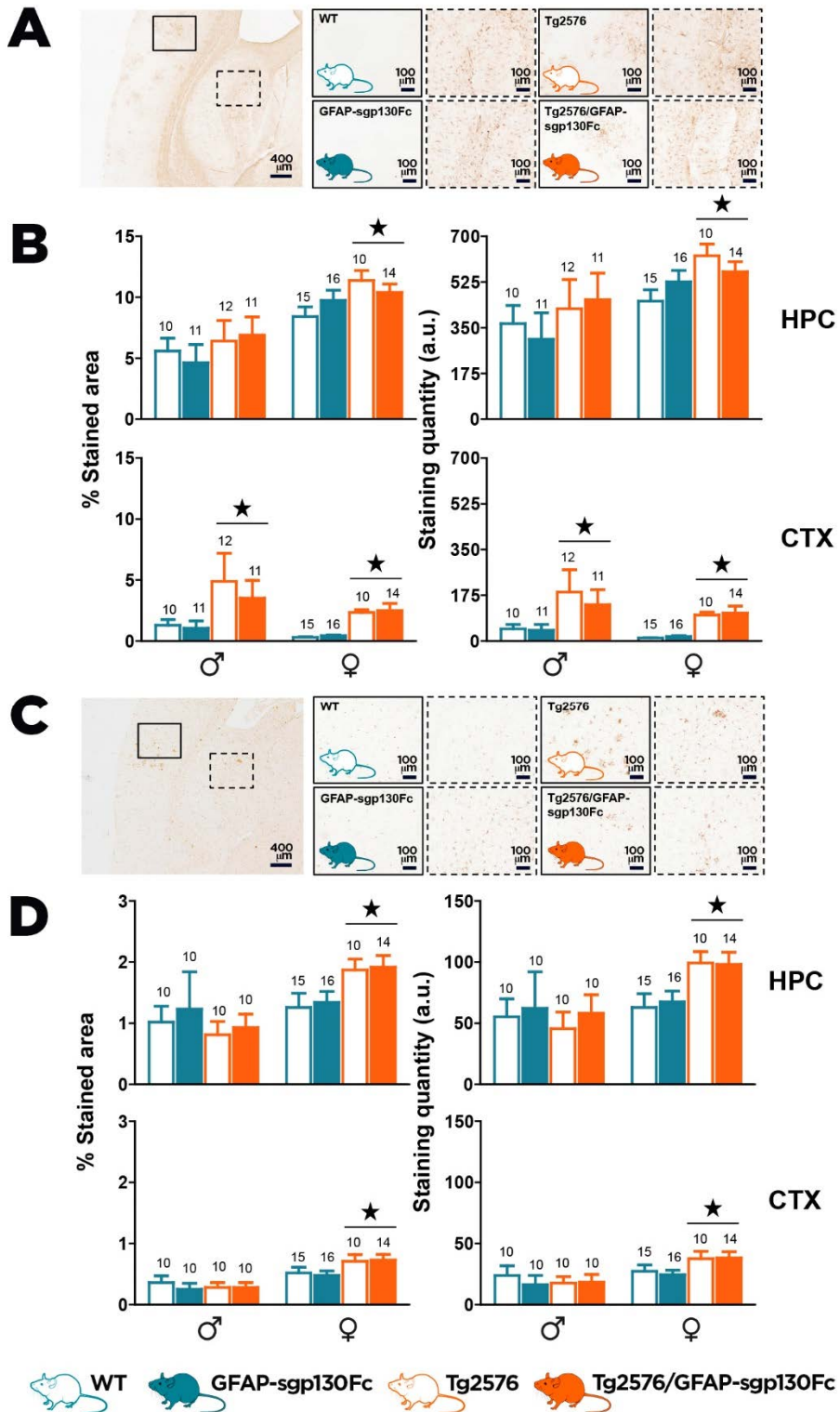
Figure 12. Effect of the inhibition of IL-6 trans-signaling on gliosis in cortex and hippocampus from ~16-19 month-old mice. (A, C) Representative immunostaining for GFAP (astrocytes) and IBA-1 (microglia), respectively (left); higher magnification of cortex (continuous line) and hippocampus (dashed line) (right). (B, D) Quantification of GFAP and IBA-1 staining, respectively. APP positive mice showed increased gliosis surrounding plaques, especially females; the inhibition of IL-6 trans-signaling did not affect these measured parameters. Results are MEAN ± SEM. ★ *p≤ 0.05 vs. APP negative.*

The inhibition of IL-6 trans-signaling reduced total Aβ$_{40}$ and Aβ$_{42}$ levels in cortex from females, whereas, no effects were observed in males or in hippocampus.

APP and its proteolytic fragments were analyzed in total homogenates of cortex and hippocampus of mice ranging from approximately 16 to 19 month old using WB (Fig. 13A) and ELISA (Fig. 13B). Regarding the amyloid cascade analysis by WB, the inhibition of IL-6 trans-signaling decreased APP in males' hippocampus. However, the opposite effect was observed in hippocampus from females, it increased APP and likely, as a consequence its subsequent proteolytic fragments, c-terminal fragment-β (CTF-β) and Aβ (monomeric). These effects were not replicated by the ELISA analysis, being a more sensitive method, but a decrease in Aβ amount was displayed by the double transgenic females in cortex. When sex is included as a factor, the total Aβ$_{42}$ amount is significantly higher in females than in males in both brain regions, the same occurred in total Aβ$_{40}$ amount in cortex whereas, both sexes maintained equal levels in hippocampus.
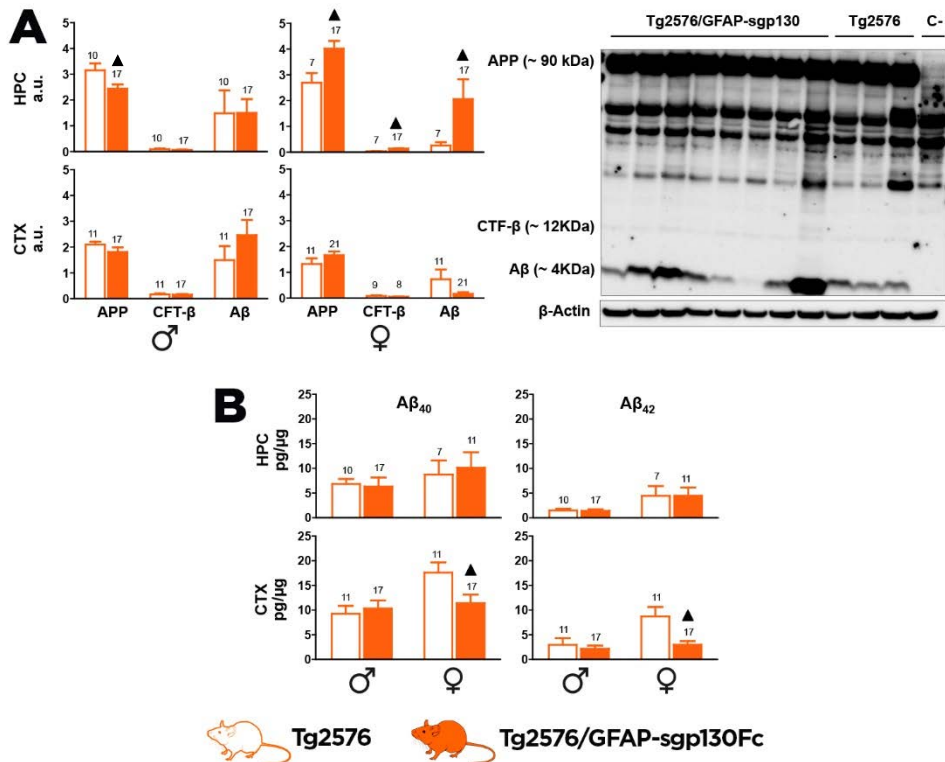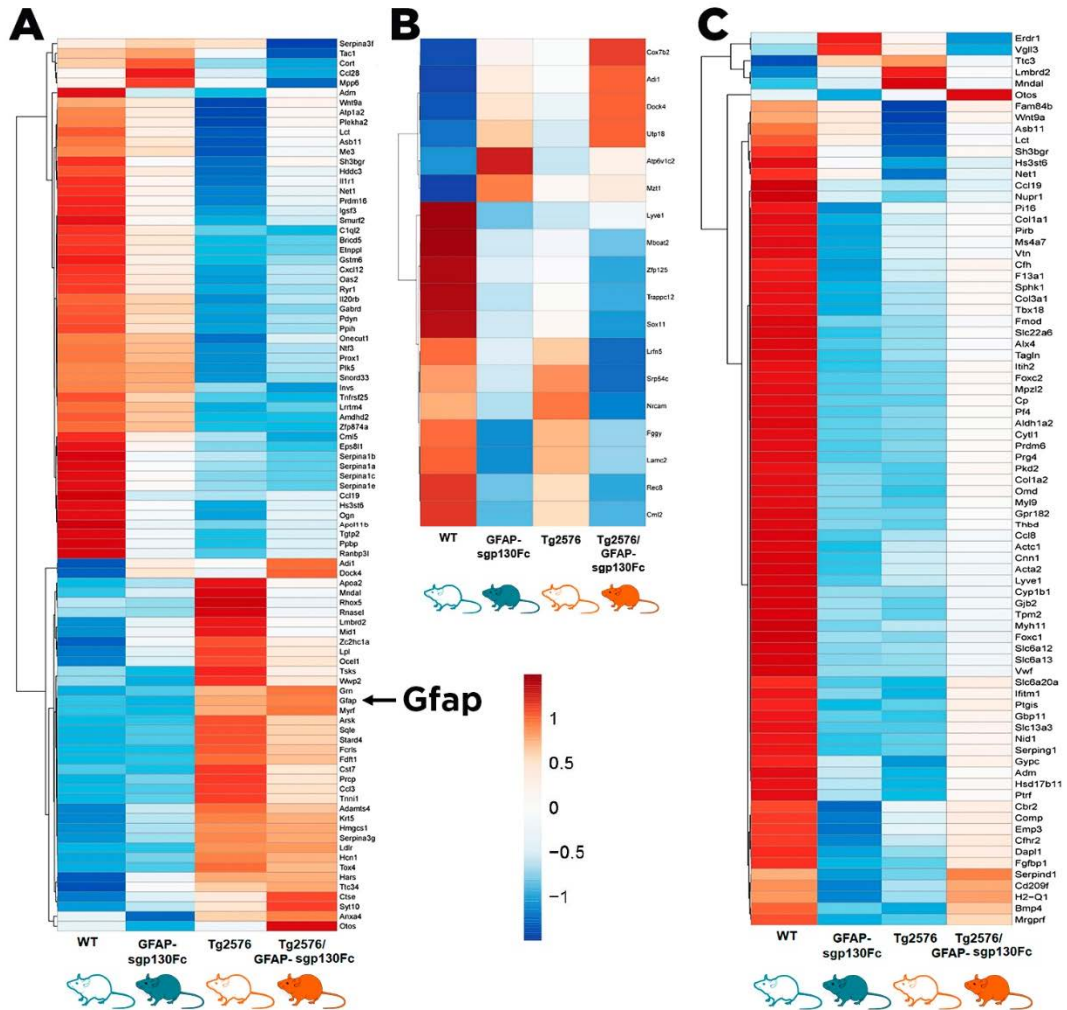
Figure 13. Effect of the IL-6 trans-signaling inhibition on amyloid cascade measured in cortex and hippocampus from ~16-19 month-old mice. (A) Full length APP and its proteolytic fragments were analyzed by western blot (right). Western blot quantification indicated that the presence of sgp130Fc reduced APP in hippocampus from males and increased APP and its proteolytic fragments in hippocampus from females (left). (B) ELISA analysis of $A\beta_{40}$ and $A\beta_{42}$ levels in both brain regions. The inhibition of IL-6 trans-signaling reduced $A\beta_{40}$ and $A\beta_{42}$ levels in cortex from females. Results are MEAN ± SEM. ▲ $p \leq 0.05$ vs. sgp130Fc negative.

The inhibition of IL6 trans-signaling affected genes involved in structure/matrix organization and blood vessels development.

Microarray analysis were performed in hippocampus from old females, as it is one of the earlier and most affected brain areas in AD. The analysis revealed differential gene expression depending on the genotype. APP, sgp130Fc and the interaction between factors were analyzed separately. When the APP effect is analyzed (Fig. 14A, 14D), the APP positive mice showed an upregulation of genes involved in processes related to

the cholesterol metabolism (i.e. *Apoa2* or *Ldlr*). Besides, as expected, the expression of *Gfap* was increased in APP positive animals. In contrast, genes involved in the regulation of peptidases were downregulated, as a great number of serpin transcripts were differentially expressed. The analysis of the sgp130Fc factor (Fig. 14B) revealed only 18 differentially expressed genes, which are not related to each other as no GO was available for these candidate gens. Finally, when the interaction between factors was analyzed (Fig. 14C, 14E), 79 genes were differentially expressed. Most of the enriched genes are related to structure/matrix organization and blood vessels development (i.e. *Col1a1*, *Col1a2*, *Myh11*).

A

Serpina3f, Tac1, Cort, Ccl28, Mpp6, Adm, Wnt9a, Alp1a2, Plekha2, Lct, Asb11, Me3, Sh3bgr, Hddc3, Il1r1, Net1, Prdm16, Igsf3, Smurf2, C1ql2, Briccd5, Etnppl, Gstm6, Cxcl12, Oas2, Ryr1, Il20rb, Gabrd, Pdyn, Ppih, Oneocut1, Nt5, Prox1, Plk5, Snord33, Invs, Tnfrsf25, Lrrtm4, Amdhd2, Zfp874a, Cml5, Eps8l1, Serpina1b, Serpina1a, Serpina1c, Serpina1e, Ccl19, Hs3st6, Ogn, Apol11b, Tgtp2, Pbbp, Ranbp3l, Adi1, Dock4, Apoa2, Mndal, Rhox5, Rnasel, Lmbrd2, Mid1, Zc2hc1a, Lpl, Ocel1, Tsks, Wwp2, Grn, Gfap ← **Gfap**, Myrf, Arsk, Sqle, Stard4, Fcrls, Fdft1, Cst7, Prcp, Ccl3, Tnni1, Adamts4, Krt5, Hmgcs1, Serpina3g, Ldlr, Hcn1, Tox4, Hars, Ttc34, Ctse, Syt10, Arxa4, Ctos

WT, GFAP-sgp130Fc, Tg2576, Tg2576/GFAP- sgp130Fc

B

Cox7b2, Adi1, Dock4, Utp18, Atp6v1c2, Mzl1, Lyve1, Mboat2, Zfp125, Trappc12, Sox11, Lrfn5, Srp54c, Nrcam, Fggy, Lamc2, Rec8, Cml2

WT, GFAP-sgp130Fc, Tg2576, Tg2576/GFAP- sgp130Fc

C

Erdr1, Vgll3, Ttc3, Lmbrd2, Mndal, Otos, Fam64b, Wnt9a, Asb11, Lct, Sh3bgr, Hs3st6, Net1, Ccl19, Nupr1, Pi16, Col1a1, Pirb, Ms4a7, Vtn, Cfh, F13a1, Sphk1, Col3a1, Tbx18, Fmod, Slc22a6, Alx4, Tagln, Itih2, Foxc2, Mpzl2, Cp, Pf4, Aldh1a2, Cytl1, Prdm6, Prg4, Pkd2, Col1a2, Omd, Myl9, Gpr182, Thbd, Ccl8, Actc1, Cnn1, Acta2, Lyve1, Cyp1b1, Gjb2, Tpm2, Myh11, Foxc1, Slc6a12, Slc6a13, Vwf, Slc6a20a, Ifitm1, Ptgis, Gbp11, Slc13a3, Nid1, Serping1, Gypc, Adm, Hsd17b11, Ptrf, Cbr2, Comp, Emp3, Cfhr2, Dapl1, Fgfbp1, Serpind1, Cd209f, H2-Q1, Bmp4, Mrgprf

WT, GFAP-sgp130Fc, Tg2576, Tg2576/GFAP- sgp130Fc

GO terms (app, up-regulated genes)

steroid metabolism, cholesterol metabolism, secondary alcohol metabolism, sterol metabolism

% Sequence
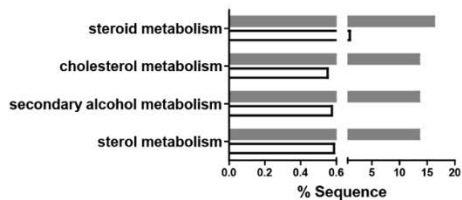
D **GO terms (app, down-regulated genes)**

negative regulation of endopeptidase activity, neg. regul. of peptidase activity, response to cytokine, neg. regul. of leukocyte tethering or rolling

% Sequence

E **GO terms (interaction)**

collagen fibril organization, extracell. matrix organization, blood vessel development, extracell. structure organization
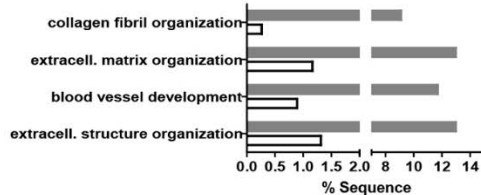
% Sequence

71
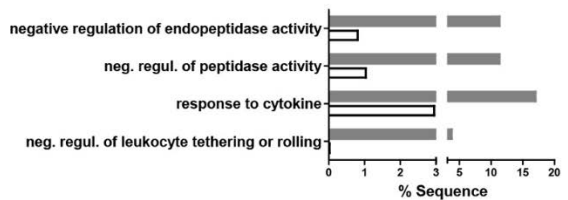
Figure 14. Microarray analysis of the hippocampus of female ~16-19 month-old mice. (A, B, C) Heatmaps of expression values for differentially expressed genes for the main factors "APP" (A) and "sgp130Fc" (B), and for the "interaction" (C). (D, E) Gene ontology clusters that were significantly enriched. (n = 4 per group).

Discussion

The role of the IL-6 in AD is still controversial. By using a new double transgenic mouse model, co-expressing hAPP and hsgp130Fc specifically in the CNS, the implication of IL-6 trans-signaling in AD pathogenesis has been demonstrated behaviorally and neuropathologically before and after the amyloid plaques deposition.

App transgene had important effects on survival. In previous studies reduced pre- and postnatal survival were reported in this mouse model (Phinney et al. 2003; Manso et al. 2012a, b, 2016), here, APP positive females showed an increased intrauterine or perinatal mortality that was not affected by the inhibition of the IL-6 trans-signaling, although IL-6 (Zolti et al. 1991) and GFAP (Brenner and Messing 1996) are expressed at embryonic stages. In this study, perinatal and/or intrauterine mortality was analyzed using the Mendelian weaning proportions, so it was not possible to know *App* transgene either affected intrauterine development or early postnatal survival rates. What is known is that APP plays an important role during embryonic development and, besides, experiments with knock-out mice for the different *App* family members (APP, APLP1 and APLP2) demonstrated also an important function during the early postnatal development (von Koch et al. 1997; Heber et al. 2000). In addition, APP positive mice from both sexes showed a higher mortality rate from weaning up to euthanasia in comparison to their APP negative littermates. The inhibition of IL-6 trans-signaling seemed to rescue partially the after weaning APP-induced mortality in both sexes, although only significantly in females. The increased mortality in the Tg2576 model was extensively described by our group (Manso et al. 2012a, b, 2016) and others (Carlson et al. 1997; Westmark et al. 2008), but, the precise reason for the lethal Tg2576 phenotype is not clear. It has been demonstrated that having different genetic background could be either beneficial (SJL/J and 129S6) or detrimental (C57Bl/6J and FVB/N) regarding the Tg2576-induced mortality (Carlson et al. 1997). Additionally, some studies have showed that the expression of Aβ induces cell death which is accompanied by seizures and premature death (LaFerla et al. 1995). More studies give support to the "seizures hypothesis", as the overexpression of APP or Aβ increased seizure susceptibility (Westmark et al. 2008; Chan et al. 2015). IL-6 has also been involved in the modulation of basal synaptic transmission in different ways; GFAP-IL6 mice, which express IL-6 under the GFAP promoter and hence overexpress IL-6 in the CNS, develop a severe neurologic disease including seizures provoked by the damage in hippocampus's inhibitory neurons (Campbell et al. 1993),

besides, the stimulation of these mice with low doses of kainic acid which have no effect in WT mice provoked seizures and death in GFAP-IL6 mice indicating an enhanced sensitivity to glutamatergic-induced seizures and lethality (Samland et al. 2003). IL-6 trans-signaling has also been implicated in the regulation of basal excitatory and inhibitory synaptic transmission (Cuevas-Olguin et al. 2017). Despite, GFAP-sgp130Fc mice presented higher sensitivity to pentylenetetrazole-induced seizures than the respective non-transgenic control group (Cuevas-Olguin et al. 2017), it is possible that the inhibition of IL-6 trans-signaling interfered differently in the present study considering that the seizures are given by different situations. In addition to seizures, vascular disruption has been pointed out as a possible cause of premature mortality (Hee et al. 2015). In agreement, our microarrays results revealed numerous DE genes involved in blood vessels development such as *Col1a1* and *Col1a2*.

A reduced body weight is observed in APP positive mice. This result is in agreement with previous studies (Manso et al. 2012a, b; Ishii et al. 2014) and it has been attributed to a dysfunction in hypothalamic leptin-responsive neurons of this model (Ishii et al. 2014). No effect of the inhibition of IL-6 trans-signaling was observed in weight, however, the IL-6 has been implicated in body weight regulation and metabolism previously; IL6-KO mice develop mature-onset obesity (Wallenius et al. 2002b), whereas, GFAP-IL6 mice are resistant to high-fat diet-induced obesity (Hidalgo et al. 2010). The central application of IL-6 in mice suppresses feeding behavior and improve glucose tolerance, particularly, IL-6 trans-signaling is enhanced in the CNS of obese mice, intensifying the IL-6 beneficial metabolic effects (Timper et al. 2017), besides, IL-6 trans-signaling mediates the macrophages recruitment into adipose tissue (ATM) in a high-fat diet-induced obesity animal model (Kraakman et al. 2015).

Some behavioral traits were analyzed before and after the amyloid plaques deposition. Contradictory results have been found previously, but hyperactivity in Tg2576 animals is widely accepted (King and Arendash 2002; Manso et al. 2012a, 2016), measured by OF young APP positive females showed an increment in activity that was maintained after amyloid plaques deposition, this same effect appeared in males at older ages. The inhibition of IL-6 trans-signaling affected this effect in a sex-dependent manner, reversing it in males and increasing activity independently from the *App* transgene in females. In a study using mice with the IL-6 trans-signaling blocked in the periphery (overexpressing sgp130Fc under PEPCK promoter), no

effects of the blockade were observed in activity in the OF (Braun et al. 2013), however, hipoactivity was observed in astrocytic-IL6 deficient mice and total IL-6 KO mice (Armario et al. 1998; Quintana et al. 2013; Erta et al. 2015). Regarding exploration and anxiety, previous studies using the Tg2576 mouse model described an enhanced exploratory behavior and reduced anxiety, related to disinhibitory tendencies that are also present in some AD patients (Lalonde et al. 2003; Manso et al. 2016). Accordingly, the present study at young ages showed an increased exploration and reduced anxiety in female mice (but not males); the inhibition of IL-6 trans-signaling partially reversed the APP-dependent effects. These results are partially in accordance with preceding investigations which showed that the blocking of IL-6 trans-signaling in the periphery reduced exploration in male mice, although no effect was observed in anxiety (Braun et al. 2013). Total IL6-KO mice reported lower time/entries in open arms than their respective controls at 2 months of age (Armario et al. 1998). In contrast, in studies using mice deficient in astrocytic-IL6 or astrocytic-IL6R it was shown a sex-dependent reduction in exploratory behavior and anxiety (~5-7 weeks), however, the opposite effects on anxiety were observed at older ages (~8 months)(Quintana et al. 2013; Erta et al. 2015). After amyloid plaques deposition, only the APP effects on exploration and anxiety were maintained, additionally, old APP positive male mice also showed reduced anxiety level in comparison with the APP negative littermates. In contrast, no sgp130Fc effect is conserved at advanced ages in these parameters.

As previously described (Hsiao et al. 1996; Manso et al. 2012a, b, 2016) APP-dependent deficits in learning and memory were showed before and after amyloid plaques deposition. The inhibition of IL-6 trans-signaling improved the performance of the old double transgenic females in the acquisition immediate probe trial, this improvement was statistically significant when the latency to arrive to the previous platform location was measured (data not shown); in this regard, the blocking of IL-6 trans-signaling significantly decreased the latency. In contrast, in the reversal immediate probe trial, the blocking of the IL-6 trans-signaling worsened the females' performance independently from the *App* transgene in young and old animals. Contradictory results have been found previously, no effect in hippocampal-dependent memory was found when peripheral IL-6 trans-signaling was blocked (Braun et al. 2013), whereas, when astrocytic-IL6 KO and total IL-6 KO mice were challenged in the MWM, deficits in learning and memory were found (Baier et al. 2009; Erta et al. 2015). In contrast, other authors described improved spatial learning (Braida et al. 2004) in IL-6 KO mice.

Full-length APP and the amyloid cascade were assessed by different techniques. APP is cleaved by β-secretase to produce CTF-β which is subsequently cleaved by γ-secretase to produce $A\beta_{40}$ or $A\beta_{42}$ depending on the cleavage site. The Aβ peptides can exist as soluble monomers, oligomers, fibrils or aggregate to form amyloid plaques (Haass and Selkoe 2007). As expected, APP positive mice from both sexes showed amyloid pathology. However, the inhibition of the IL-6 trans-signaling only had major effects in females which showed higher Aβ levels than males. Gender differences were previously described by Callahan and colleagues, aged females had augmented plaque load as well as $A\beta_{40}$ and $A\beta_{42}$ levels compared to males, mimicking the higher AD prevalence in women. Even though the reason for the increased pathology is not clear, it could be attributed to the effects of estrogen to Aβ processing (Callahan et al. 2001; Allué et al. 2016). The inhibition of IL-6 trans-signaling increased APP and likely, as a consequence, CTF-β and Aβ levels in hippocampus by western blot. This increment in hippocampal Aβ was not observed in a specific Aβ ELISA. In fact, both $A\beta_{40}$ and $A\beta_{42}$ levels were diminished in cortex from the double transgenic females. In line, double transgenic females had reduced plaque burden in both, cortex and hippocampus, in comparison with the Tg2576 animals, suggesting a role of IL-6 trans-signaling in the Aβ aggregation process. Surprisingly, the diminished plaques deposition did not correlate with diminished gliosis, in accordance, no DE genes involved in inflammation were detected in interaction between factors in the microarray analysis from females' hippocampus. In males, the inhibition of IL-6 trans-signaling decreased APP in hippocampus when measured by WB. No changes in amiloidosis were observed in cortex by WB, or in both regions by ELISA or IHC.

As far as we know, no studies about IL-6 trans-signaling and AD have been performed in mice, nevertheless, conflicting results have been found in AD patients. In a recent study was found that a common variant of IL-6R which increases sIL-6R levels, in periphery and CNS, is associated with an earlier age of AD onset in APOE4+ cases. In the same study, it was observed an enrichment in astrocyte derived IL-6 responsive genes in LOAD patients with increased sIL-6R, thus, increased trans-signaling pathway. One of these genes was *Serpina3*, which is in line with our microarray results and with the previously described by Campbell et al. (Campbell et al. 2014), postulating that an enhanced trans-signaling and a reduced cis-signaling (classic signaling) particularly driven by astrocytes increase amyloid levels and advance the age of onset in APOE4+ patients (Haddick et al. 2017).

The way sgp130Fc interfered with Aβ deposition is unknown. Microarray analysis revealed enrichment in genes related to structure or matrix organization and blood vessels development. Components of the extracellular matrix as proteoglycans or glycosaminoglycans could affect aggregation, intracellular internalization or clearance of Tau and Aβ (Cui et al. 2013). They also regulate APP processing, as well as, Aβ fibril and amyloid plaques formation (Castillo et al. 1997; Sethi and Zaia 2017). Moreover, vascular dysfunction as morphological changes in cerebral arteries, cerebral hypoperfusion and impaired Aβ clearance through the blood-brain barrier has been observed in AD patients and AD animal models, including Tg2576, and plays an important role in AD pathogenesis (Bell and Zlokovic 2009; Klohs et al. 2014). Previous studies has shown that the cerebrovascular endothelium is highly responsive to IL-6 and that the inhibition of IL-6 trans-signaling in the CNS significantly reduced vascular alteration and BBB leakage in GFAP-IL6 mice (Campbell et al. 2014). These could be possible explanations for the reduced mortality and reduced Aβ accumulation in double transgenic female mice, but more investigation is needed in this regard.

Conclusions

The implication of IL-6 trans-signaling in AD pathogenesis has been demonstrated in this study. IL-6 trans-signaling inhibition affected mortality, behavior (before and after the amyloid plaques deposition) and Aβ levels and accumulation, especially in females. Further investigation is needed to unravel the precise mechanism. Some limitations should be mentioned, the high mortality of APP positive animals made difficult to achieve a sufficient number. The animals were analyzed in age-matched groups as the animals grew, thus increasing the variability inter-groups. Additionally, a narrower range of age should be analyzed as the variability between animals, especially in Aβ quantification and plaque load, was pretty high to extract more accurate conclusions. Nowadays, the sgp130Fc protein is in clinical trials phase II for inflammatory diseases as ulcerative colitis and inflammatory bowel disease (Garbers et al. 2018), if they become favorable, the application of sgp130Fc in AD should be considered.

CHAPTER 2

IL-6 trans-signaling in the brain influences the behavioral, physio-pathological and metabolic phenotype of the 3xTg-AD mouse model of Alzheimer's disease.

Abstract

In this Chapter, we have addressed the possibility that blocking IL-6 trans-signaling in the brain could have an effect in the triple transgenic 3xTg-AD mouse model of AD and/or in obesity progression, by crossing homozygous 3xTg-AD mice with heterozygous GFAP-sgp130Fc mice. In our experimental conditions, the 3xTg-AD model showed a mild amyloid phenotype, which nevertheless altered life span, some behavioral traits, learning and memory. It also affected the control of body weight with both a control and a high-fat diet (HFD), and related endocrine and metabolic factors. The inhibition of IL-6 trans-signaling modulated some of these traits in both 3xTg-AD and control mice, in a sex-dependent manner.

Material and methods

Generation of double transgenic 3xTg-AD/GFAP-sgp130Fc mice

The parental strains used in this study, 3xTg-AD mice (provided by Dra. Lydia Giménez-Llort) and GFAP-sgp130Fc (provided by Dr. Stefan Rose-John), were generated and characterized previously (Oddo et al. 2003b; Campbell et al. 2014). To obtain the 4 genotypes of interest the following crossing strategy was designed. Homozygous 3xTg-AD (+/+) animals were mated with heterozygous GFAP-sgp130Fc (+/-) to obtain the double transgenic 3xTg-AD/GFAP-sgp130Fc (+/-) (+/-) and 3xTg-AD (+/-) (-/-) mice. In addition, GFAP-sgp130Fc (+/-) mice were also crossed with WT mice (C57BL/6JOlaHsd) to obtain the AD negative controls GFAP-sgp130Fc (-/-) (+/-) and WT (-/-) (-/-). Genotype was determined by PCR analysis of tail DNA (see supplementary Material and Methods).

All mice used in this study were fed *ad libitum* (unless otherwise stated) and housed in a 12-hour dark-light cycle under specific pathogen-free conditions and constant temperature (22 ± 2ºC). All experimental procedures were approved by the Ethics Committee in Human and Animal experimentation (CEEAH 3971) from the Autonomous University of Barcelona and were in accordance with Spanish legislation and the EU directive (2010/63/UE) on 'Protection of Animals Used for Experimental and Other Scientific Purposes'. The study complies with the ARRIVE guidelines developed by the NC3Rs.

After weaning at 3 weeks of age, body weight and mortality were controlled regularly up to euthanasia. Some animals were killed at early ages for neuropathological analysis, but in most cases they were killed when >20 months of age. Animals were killed by decapitation to collect the brain. The left hemisphere was immersed in 4% paraformaldehyde (PFA) for 24 hours and stored in 70% ethanol at 4ºC until paraffin inclusion for immunohistochemistry (IHC). The right hemisphere was dissected into cortex, hippocampus and in some experiments hypothalamus and flash-frozen in liquid nitrogen for gene expression, Western Blotting and ELISA. Blood was collected from the trunk and centrifuged (9520 g for 10 minutes) at 4ºC to obtain the serum. All samples were stored at -80ºC until processed. Subsets of animals were killed at different ages to check disease progression.

Diets

A subset of animals was challenged with a high-fat diet (HFD). Both the HFD (TD 03584, 58.4% kcal from fat), and the control diet (2018, 18% kcal from fat) were obtained from Harlan Iberica. The experiment had two phases and began when the mice were 17 month old: they were first fed the control diet (n=6-11); and then the HFD. Because of aging-related mortality in AD male mice, 8 new animals were added in the second phase. Body weight and food intake were monitored regularly. In most cases mice were not isolated but kept group-housed after weaning (2–3 per cage) according to their genotype and sex. Body temperature was measured at the beginning of the control diet phase. Animals were subjected to a fasting and refeeding test (F&R), an insulin tolerance test (ITT) and an oral glucose tolerance test (OGTT), the first was carried out during the control diet, the other two in both phases. Hypothalamus, liver, visceral (gonadal) and subcutaneous (inguinal) fat depots and brown adipose tissue were removed and weighed upon euthanasia of the animals and frozen in liquid nitrogen for further analysis.

Behavioral tests

The same behavioral tests than in Chapter 1 were carried out, but in this case mice were tested at ~5-6 and at ~10-11 months of age.

Open Field Test (OF)

Mice were placed in the corner of a rectangular methacrylate white box (56 x 36.5 x 31 cm) and allowed to freely explore the apparatus for 5 minutes. The measured parameters were: total distance moved (horizontal activity), rearings (vertical activity) and defecations.

Hole-Board Test (HB)

Mice were placed in the corner of an apparatus consisting of a square white methacrylate box (40 x 40 x 21.5 cm) with four equidistant holes and allowed to freely explore it for 5 minutes. The measured parameters were: time and number of head-dipping (HD) events (considered to be when an animal inserted the head into the holes at least to its ear level), total distance moved (horizontal activity) and defecations.

Elevated-plus Maze Test (EPM)

The EPM apparatus is a white elevated maze with four arms (20 x 5 cm), two open and two closed (15 cm high wall), connected by a central square (5 x 5 cm). The apparatus is elevated 50 cm from the floor. Mice were placed in the central square facing the closed arms and left to explore for 5 minutes. Entries and time in closed and open arms were measured. For analysis, the percentage of "time spent in" or "entries to" open arms was calculated (as the percentage from total time or entries to both open and closed arms, excluding the center).

Spatial learning and memory: Morris water maze (MWM)

The MWM consisted of a propylene round pool (120 cm in diameter and 60 cm deep) virtually divided in four equal quadrants and a removable platform. It contains opaque (by adding white tempera) water at $22 \pm 1°C$ and four 2D cues placed on the pool walls and four 3D cues placed before the surrounding curtain. The animals were assessed in three learning and memory paradigms (cued, place task and reversal). They were first exposed to a cued-learning session using a visible platform in order to check quadrant preferences or visual alterations. The following 4 days, in the place task acquisition, mice had to escape from the pool by finding a hidden platform in a target quadrant (TQ) (submerged 1 cm below opaque water) using the cues as spatial references. Animals were freely allowed to explore the pool for 60 seconds and, in case they failed to find the platform, they were guided to it and required to remain on it for 15 seconds more. Animals were trained for 4 consecutive days with four trials per day and the escape latency (time required to localize the hidden platform) in each trial was recorded (~1 hour inter-trial). After this phase, short and long-term memory were tested by means of probe trials administered ~1 hour after the last training session (day 5) and 24 hours later (day 6), respectively. These tests consisted in removing the platform and measuring the time the mice spent in the TQ of the pool (the one where the hidden platform was located) out of a 30-second trial.

From day 7 to 10, mice were made to learn a new platform location in the reversal paradigm, when the location of the hidden platform was changed to the opposite quadrant. As before, acquisition was followed by two probe trial tests for short and long term memory (days 10 and 11).

Analysis of behavior

The data from the behavioral tests was obtained through both, *in situ* manual measurements (rearings, defecations and HD) and automated tracking analysis using specific software: ANY-Maze version 5.14 for the MWM test and EthoVision XT version 11.5 (Noldus) for the rest of them.

Fasting and Refeeding (F&R)

The animals were fasted overnight for 14 hours and posteriorly refed. Body weight and food intake were measured regularly during the first 48 hours post-fasting. Using these parameters body weight change post-fasting was calculated as well as the food intake normalized to body weight.

Body temperature

Body temperature measurement was carried out using a lubricated rectal probe (Cibertec). The measurements were taken throughout the day, at 8, 11, 16 and 19 hours.

Insulin Tolerance Test (ITT) and Oral Glucose Tolerance Test (OGTT)

The metabolic status of the mice was evaluated by an insulin tolerance test (ITT) and an oral glucose tolerance test (OGTT). For ITT, mice were injected intraperitoneally (i.p.) with 1 U/kg of insulin after a 4-hour fast. For OGTT, mice were fasted for 15 hours before being administered an oral glucose dose of 2 g/kg of body weight. Blood glucose was measured in tail samples at time points 0, 15, 60 and 120 min for ITT and at 0, 30, 60 and 120 min for OGTT with an ACCU CHECK glucometer (Roche).

Western Blotting for Amyloid cascade and sgp130Fc detection

Amyloid cascade was analyzed as described in Chapter 1.

For detection of sgp130Fc, brain samples were homogenized using a Precellys tissue homogenizer in RIPA buffer supplemented with phosphatase and protease inhibitors.

Brain homogenates were incubated with 100 μl protein A Sepharose (50% diluted in PBS) overnight at 4°C for immunoprecipitation. Western-blot was performed essentially as described previously (Campbell et al. 2014). In this case, GAPDH (14C10, 1:10000, Cell Signaling) was used as loading control.

Enzyme-Linked ImmunoSorbent Assays (ELISAs) and colorimetric tests

ELISA kits were used to measure $A\beta_{40}$, $A\beta_{42}$ and sgp130Fc levels in brain samples (homogenized as in WB section of Chapter 1 and WB section of Chapter 2) (Amyloid beta 40 and 42 Human ELISA Kits, ThermoFisher; Human sgp130 DuoSet ELISA, R&D systems) and leptin, insulin and FGF-21 in serum (rat/mouse ELISA leptin/insulin kits, Millipore; Mouse/Rat FGF-21, R&D systems).

To measure cholesterol, triglycerides and glucose in serum specific colorimetric test kits were used (Cholesterol MonlabTest®, Triglycerides MonlabTests® and Glucose-TR Spinreact).

Immunohistochemistry (IHC)

IHC analyses was carried out as described in Chapter 1.

RT-qPCR

For the two-step RT-qPCR, total RNA was isolated using Maxwell simply RNA Tissue Kit (Promega) from hypothalamus and reverse transcribed into cDNA in a reaction using a first-strand cDNA synthesis kit, SuperScript III Reverse transcriptase according to manufacturer's instructions (Invitrogen). RT-qPCR for selected genes (*Il-6, Agrp, Mac1, Gfap and Gapdh*) was performed in 384-well plates and run in CFX384 real-time PCR detection system (Bio-Rad). (*Il6*Fw: 5'-gcttaattacacatgttctctgggaaa-3'; *Il6*Rv: 5'-caagtgcatcatcgttgttcatac-3'/ *Agrp*Fw: 5'-agagttcccaggtctaagtctg-3'; *Agrp*Rv: 5'-gcggttctgtggatctagc-3'/ *Mac1*Fw: 5'-gggaggacaaaaactgcctca-3'; *Mac1*Rv: 5'- acaactaggatcttcgcagcat-3'/ *Gfap*Fw: 5'-ggggcaaaagcaccaaagaag-3'; *Gfap*Rv: 5'- gggacaacttgtattgtgagcc-3'/ *Gapdh*Fw: 5'-ggcaaattcaacggcaca-3'; *Gapdh*Rv: 5'- cggagatgatgacccttt-3').

Statistics

Statistical calculations were done using the Statistical Package for Social Sciences (SPSS, version 19). Males and females were analyzed separately. Most data were analyzed using the Generalized Linear Model (GLZ) with 3xTg-AD (3xTg-AD positive vs. 3xTg-AD negative, AD) and sgp130Fc (sgp130Fc positive vs. sgp130Fc negative, sgp) as main factors. Generalized estimated equations (GEE) were used for repeated measures (i.e., body weight, escape latencies in MWM, ITT and OGTT) including also both genotypes as main factors and time as a within-subject factor. In case of comparisons between two groups, the t-Student was used. Survival was analyzed using the Kaplan–Meier and Log rank survival tests, using genotype as a factor with four levels (WT, GFAP-sgp130Fc, 3xTg-AD, 3xTg-AD/GFAP-sgp130Fc). Statistical significance was defined as $p \leq 0.05$.

Results

3xTg-AD positive mice showed decreased survival and body weight. The inhibition of IL-6 trans-signaling had a sex-dependent effect on survival and no-effect on body weight.

In both male and female mice, a clear trend in 3xTg-AD positive animals to increased mortality was observed. The inhibition of IL-6 trans-signaling showed opposed effects in males (detrimental) and females (beneficial) in 3xTg-AD positive mice (Fig. 15A), although pair-wise Kaplan-Meier analyses revealed significant differences only when the double transgenic male mice were compared with WT and GFAP-sgp130Fc mice, and in 3xTg-AD female mice when compared with GFAP-sgp130Fc mice (Fig. 15A).

At advanced ages (between 17 and 28 months old) and in both sexes, 3xTg-AD positive animals had lower weight than controls (Fig. 15B); the inhibition of IL-6 trans-signaling did not affect body weight.
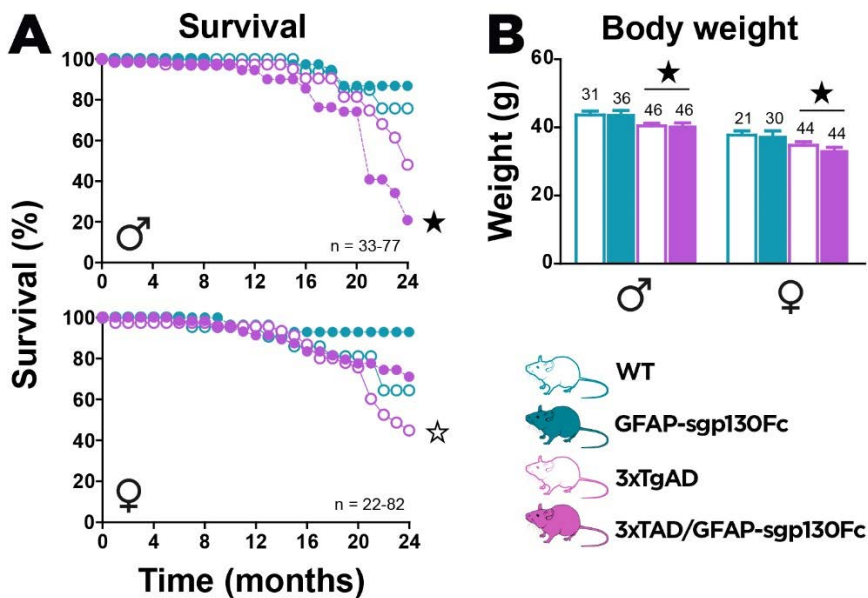

Figure 15. 3xTg-AD positive mice showed decreased survival and body weight. (A) Kaplan-Meier analysis revealed that 3xTg-AD positive mice tended to have increased mortality rate. The presence of sgp130Fc had a sex-dependent effect, increasing mortality in the 3xTg-AD/GFAP-sgp130Fc male mice when compared with WT and GFAP-sgp130Fc mice and rescuing it in 3xTg-AD/GFAP-sgp130Fc females. (B) Body weight at 17 to 28 months of age was decreased in 3xTg-AD positive animals in comparison with 3xTg-AD

3xTg-AD positive mice showed some alterations in behavioral traits that were only mildly affected by the inhibition of IL-6 trans-signaling.

At ~5-6 and ~10-11 months of age (Fig. 16) the animals were assessed in the open-field test (OF), hole-board (HB) and elevated-plus maze (EPM), three tests involving different locomotor, exploratory and anxiogenic conditions. At ~5-6 months (Fig. 16A) there was no IL-6 trans-signaling blocking effect in any of the measured parameters. No 3xTg-AD negative animals were included in this preliminary attempt to analyze behavioral changes.

At ~10-11 months there was no significant effect of the AD transgenes or the inhibition of IL-6 trans-signaling on the total horizontal activity in OF (Fig. 16B left). However, the time course of horizontal activity (Supplementary Figure 1A) revealed that in the first minute 3xTg-AD positive female mice had a decreased activity ($p<0.001$) and that sgp130Fc had a clear trend to increase activity regardless of AD transgenes ($p=0.053$). A decrease in rearings was observed in 3xTg-AD positive females, whereas an increase in defecations occurred in both sexes; and the blocking of IL-6 trans-signaling had no-effect in these two parameters.

In the HB (Fig. 16B middle), a clear AD-dependent increase on exploration (both nº and time of head-dips) was observed in both sexes. Activity, in line with the OF, was unaffected by AD transgenes. No-effect of the inhibition of IL-6 trans-signaling was seen in the measured parameters. Defecations were similar to those in the OF (data not shown). When the horizontal activity in OF and HB was considered together, we observed again no significant effects of the AD transgenes or the inhibition of IL-6 trans-signaling (data not shown), although the response in the HB was significantly modified by the previous exposure to the OF in male mice (effect of test $p<0.006$). In contrast, when the ratio OF/HB was calculated, a significant increasing effect by sgp130Fc was observed in female mice (Supplementary Figure 1B).

In the EPM (Fig. 16B right), AD positive females showed increased number of entries in the open arms, although this did not translate into time spent in these arms. There was also a trend for the inhibition of IL-6 trans-signaling to decrease this AD-related effect. In males, no effect of AD transgenes was evident, but, surprisingly, blocking of

IL-6 trans-signaling decreased anxiety, as clearly shown by the significant increase in the percentage of time spent in the open arms. Defecations were in line with the previous tests (data not shown).
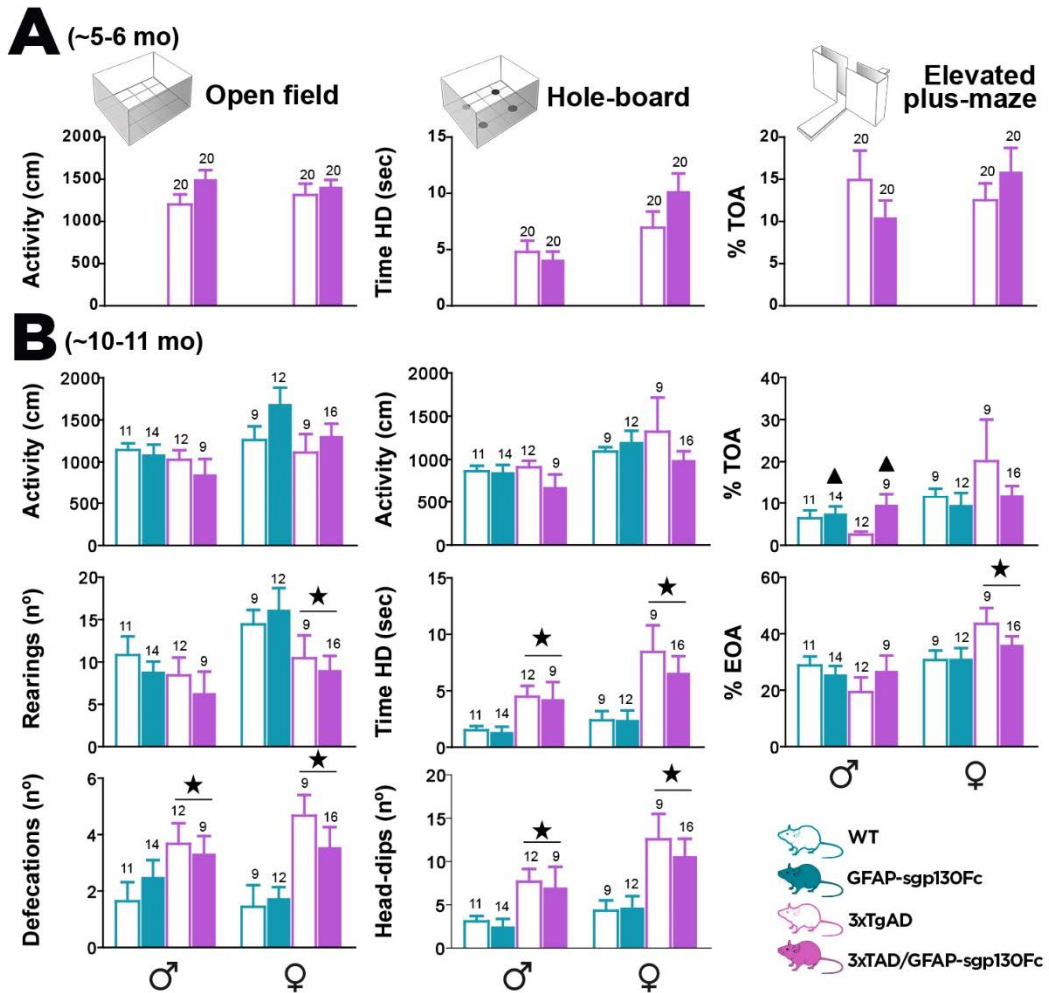

Figure 16. Behavioral characterization at early and middle ages. (A) No effect of the IL-6 trans-signaling blocking was observed at ~5-6 months in activity, exploration and anxiety. (B) In OF, at ~10-11 months, 3xTg-AD positive females showed a decrease in vertical activity and, an increase in defecations was observed in both sexes. In the HB, 3xTg-AD positive mice had exacerbated exploratory behavior showed as an increment in number and time of head-dips (HD). In the EPM, a higher number of open arms entries (EOA) in the 3xTg-AD positive females were observed; the presence of the sgp130Fc resulted in an increase in time spent in open arms (TOA) in males. Results are MEAN ± SEM. ★ $p \leq 0.05$ vs. 3xTg-AD negative; ▲ $p \leq 0.05$ vs. sgp130Fc negative.

In the MWM, ~5-6-months-old (Fig. 17A) double transgenic female mice reached the hidden platform faster in the place task acquisition phase, and spent more time in the TQ during the immediate (1 hour) probe test, than 3xTg-AD females. No differences were obtained in the reversal test (data not shown). At ~10-11 months (Fig. 17B top), there was a clear AD effect in the place task spatial acquisition in both sexes, with 3xTg-AD positive animals having a better outcome in the task. Since the distance moved did not differ between AD positive and negative animals, we expected a higher swimming speed in the former, as indeed happened; this was also the case in the cued task acquisition (Supplementary Figure 2A). Escape latencies were increased by the presence of sgp130Fc in females regardless of AD transgenes (Fig. 17B, top left), as were the distances travelled, whereas their swimming speeds were not altered (Supplementary Figure 2A). These effects were not clearly replicated in the probe trials but an interaction emerged in the long-term memory probe trial (24 hours), since in 3xTg-AD females the blocking of IL-6 trans-signaling decreased the time in the target quadrant. A similar pattern was observed in the reversal test, in which 3xTg-AD positive mice reached the hidden platform faster than 3xTg-AD negative mice (Fig. 3B bottom), likely because of their higher swimming speed (Supplementary Figure 2B). However, in this case, blocking of IL-6 trans-signaling decreased or increased the time in the target quadrant in the 24 hours probe trial in 3xTg-AD negative and positive females, respectively.

Acquisition

A (~5 mo)

B (~10 mo)

Reversal

WT

GFAP-sgp130Fc

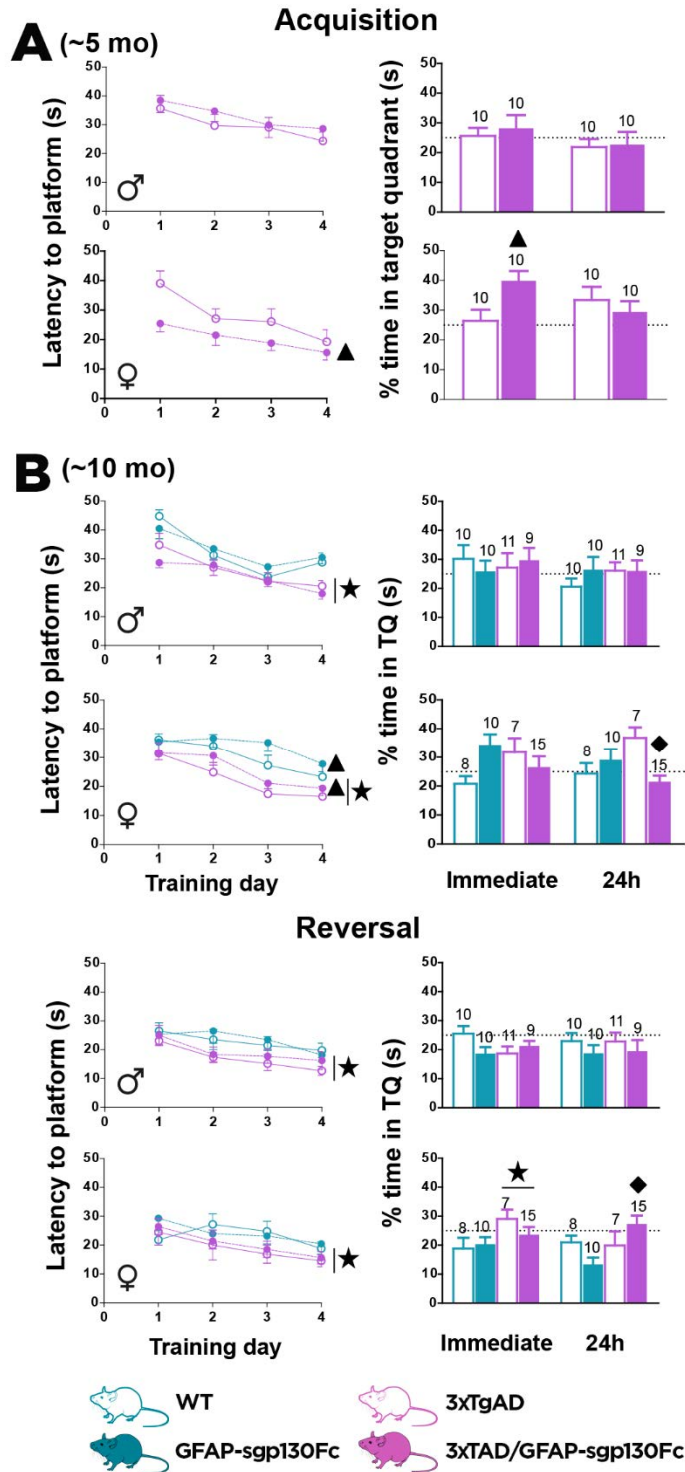3xTgAD

3xTAD/GFAP-sgp130Fc

Figure 17. The inhibition of IL-6 trans-signaling improved the MWM performance in young females, whereas the opposite effects were observed at more advanced ages. (A) At ~5-6 months, in females the presence of the sgp130Fc decreased the escape latencies to achieve the platform in the acquisition phase and increased the time spent in the target quadrant (TQ) in the immediate probe trial. (B) At ~10-11 months, 3xTg-AD positive animals from both sexes showed decreased escape latencies than controls in both test phases, this fact was not supported by a better performance in the majority of the probe trials. The presence of sgp130Fc increased escape latencies independently from the presence of AD transgenes in females. Results are MEAN ± SEM. ★ *p≤ 0.05 vs. 3xTg-AD negative;* ▲ *p≤ 0.05 vs. sgp130Fc negative;* ♦ *Indicates a significant interaction between both genotypes*

3xTg-AD/GFAP-sgp130Fc animals produced sgp130Fc in the brain. 3xTg-AD positive mice presented a mild form of AD at advanced ages.

The presence of sgp130Fc was determined in brain cortex from 3xTg-AD and 3xTg-AD/GFAP-sgp130Fc mice at 10 months of age (n=4-5), using WB (Fig. 18A left) and ELISA (Fig. 18A right). A clear increase in sgp130Fc immunostaining was observed in the 3xTg-AD/GFAP-sgp130Fc mice, with no differences between males and females.

A WB including samples from hippocampus of female mice at different ages (~5, ~10 and 20-24 months) was carried out in order to detect APP and the subsequent proteolytic fragments, CTF-β (~12 KDa) and Aβ (~4 KDa), in the 3xTg-AD positive animals (Fig. 18B). An increase in APP was observed between 5 and 10 months of age, staying constant until 24 months. No differences were observed between animals with inhibition of the IL-6 trans-signaling and animals with the preserved pathway. It was almost impossible to detect CTF-β or Aβ in the 3xTgAD or 3xTgAD/GFAP-sgp130Fc animals, but ability of the antibody to do so was confirmed with a positive control, an 18-month old Tg2576 mouse (an animal that carries the Swedish mutation).
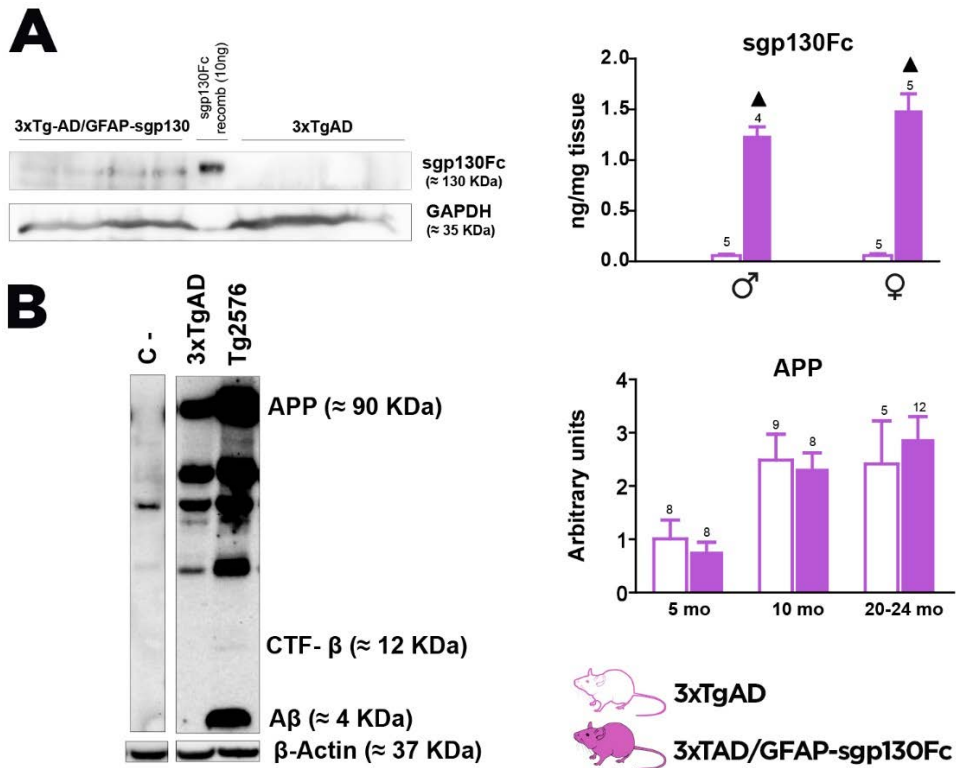
Figure 18. Presence of sgp130Fc and human APP in brain of 3xTg-AD positive mice. (A) Sgp130Fc detection and quantification by WB (left) and ELISA (right), respectively, in brain cortex of 3xTg-AD positive mice. (B) APP, CTF-β and Aβ analysis in hippocampus from 3xTg-AD positive females at different ages; no CTF-β or Aβ was detected by WB in the majority of the samples. APP was increased between 5 and 10 months of age, and then it was stabilized. Results are MEAN ± SEM. ▲ *p≤ 0.05 vs. sgp130Fc negative.*

Sgp130Fc influenced body weight and food intake in a sex-dependent manner in response to a high-fat diet.

During the control diet phase, 3xTg-AD positive animals had lower body weight in both sexes (Fig. 19A). The inhibition of IL-6 trans-signaling did not have a significant effect in this regard, although GFAP-sgp130Fc mice seemed to respond poorly to the experimental manipulations they were going through this phase compared to WT mice. Food intake (g/day) was significantly increased in 3xTg-AD positive animals, as was the case following normalization by their body weight (g/bw/day). A significant interaction between factors revealed that the inhibition of IL-6 trans-signaling in male mice significantly blunted body weight-normalized food intake of 3xTg-AD mice. In females, however, an interaction was observed in absolute food intake, because of the different effects of sgp130Fc expression (inhibiting and having no effect on food intake in 3xTg-AD negative and positive mice, respectively).

Despite their advanced age (17 months old), in general mice clearly gained weight during the HFD phase (Fig. 19B left). The same AD-effect on body weight observed in the control phase was seen in the HFD phase in both sexes. Also, the inhibition of IL-6 trans-signaling did not have a significant effect in male mice; in contrast, in females it decreased HFD-induced body weight gain. Results for food intake were similar to those of the control diet phase, except that sgp130Fc expression no longer decreased the effect of AD in male mice.

The weights of liver and fat tissues (Fig. 19B right) are in concordance with those of body weight. For the subcutaneous and visceral white adipose tissue (WAT), brown adipose tissue (BAT) and liver, the presence of the AD transgenes decreased the mass of these tissues in both sexes; in females, the inhibition of IL-6 trans-signaling also resulted in lower weight in almost all the tissues, but no trans-signaling effects were observed in males.
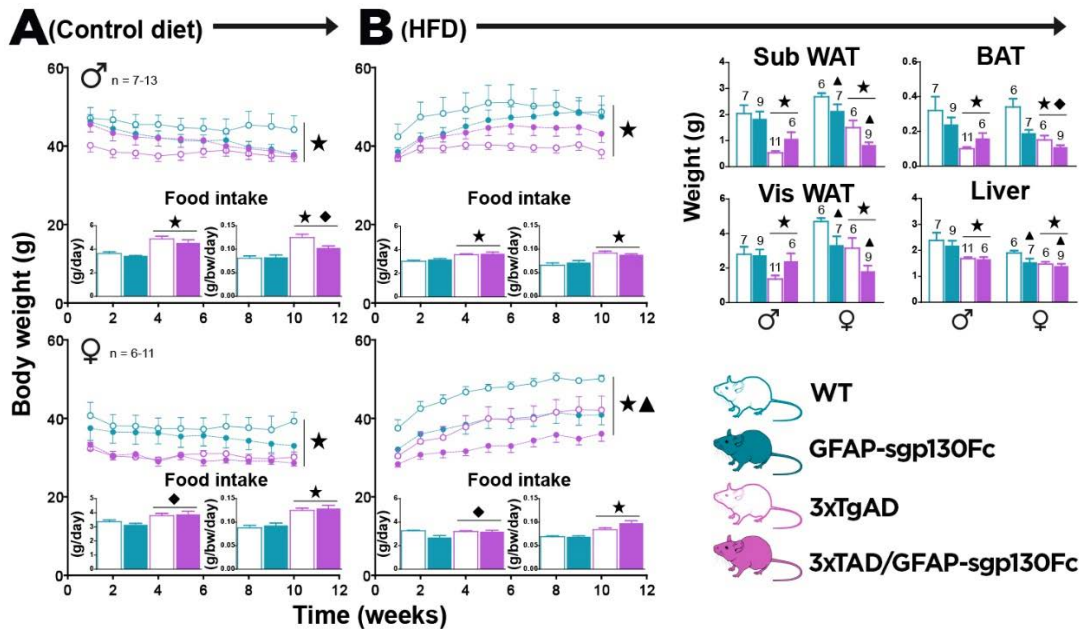
Figure 19. Body weight and food intake alterations in control and high-fat diet.
(A) During the control diet phase, 3xTg-AD positive mice presented lower body weight and increased food intake; IL-6 trans-signaling inhibition decreased food intake in the 3xTg-AD/GFAP-sgp130Fc males, whereas, in females, decreased intake in GFAP-sgp130Fc, having no impact in the 3xTg-AD positive animals (B) During the HFD phase the same AD effect in body weight and food intake than in the control phase was observed, additionally, sgp130Fc decreased body weight gain in females independently from the AD transgenes. In this case, no effect of the blocking of IL-6 trans-signaling was observed in male's food consumption, whereas the effect in females was maintained. The absolute weight from the adipose tissue depots and liver was diminished in the 3xTg-AD positive mice. The blocking of IL-6 trans-signaling diminished even more the absolute tissues weight in females. Results are MEAN ± SEM. ★ $p \leq 0.05$ vs. 3xTg-AD negative; ▲ $p \leq 0.05$ vs. sgp130Fc negative; ♦ Indicates a significant interaction between both genotypes.

3xTg-AD positive mice recovered the body weight quicker and had a higher food intake after the fast. The inhibition of IL-6 trans-signaling attenuated these effects especially in 3xTg-AD males.

After the fast, all animals predictably lost weight (Figure 20A). This loss was higher in 3xtg-AD positive mice. Once the animals were re-fed their body weight increased, with the 3xTg-AD positives being the first to recover their weight, in both sexes. Blocking IL-6 trans-signaling decreased body weight recovery in 3xTg-AD mice,

especially in males (Fig. 20A). Absolute food intake measurements (Fig. 20B left) showed a compensatory hyperphagia in 3xTg-AD mice after fasting, evident in both sexes, with sgp130Fc decreasing this AD-related response in male mice, and to a limited extent in female mice. Trends similar to those described above were observed in food intake normalized to body weight (Fig. 20B right).

Regarding body temperature (Fig. 20C), it oscillated according to the circadian rhythm between 1-3 °C throughout the day. The higher temperatures were observed in the earlier hours of the day when it was more evident that 3xTg-AD positive mice showed higher body temperature compared with 3xTg-AD negative in both sexes. However, only 3xTg-AD positive males kept the same trend throughout the day. Blocking IL-6 trans-signaling did not cause any effect.
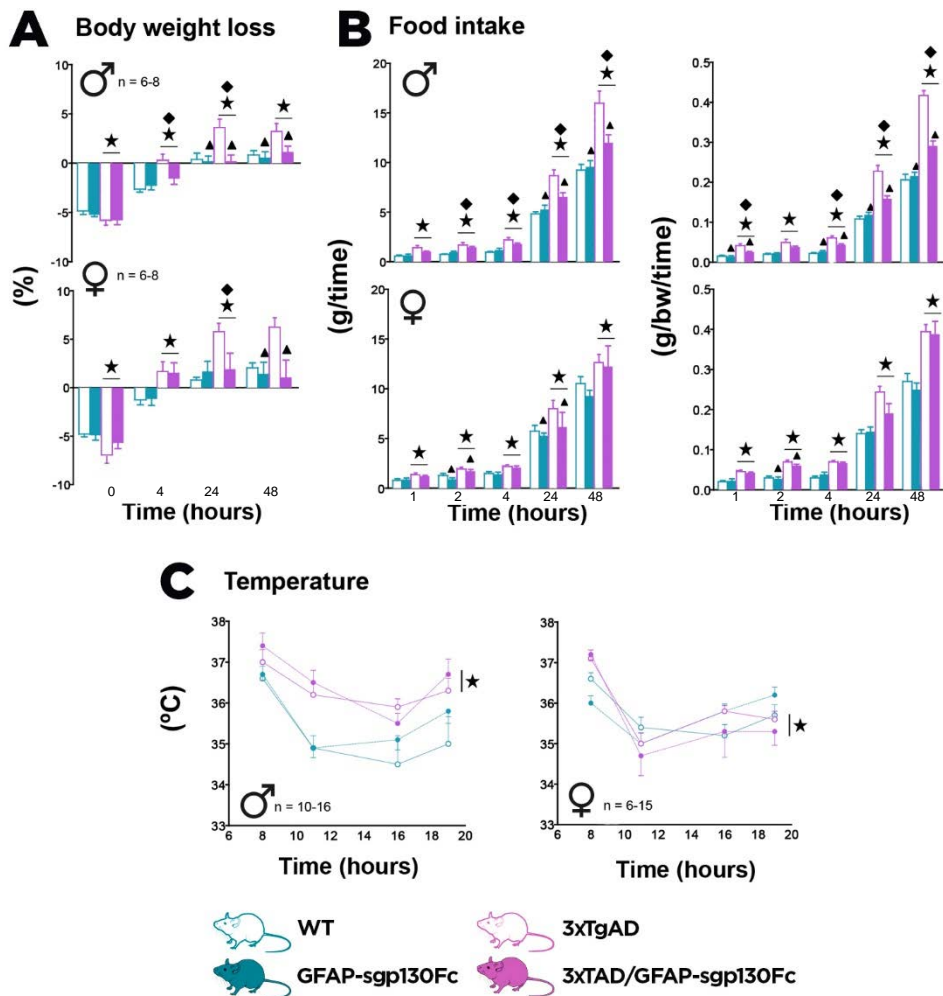
Figure 20. Fasting and refeeding analysis and basal temperature in mice fed with control diet. (A) After fasting, 3xTg-AD positive mice lost more weight than 3xTg-AD negative, without effects of the IL-6 trans-signaling inhibition. Once the animals were refed, 3xTg-AD positive animals gained weight quicker than 3xTg-AD negative; the presence of sgp130Fc blocked this effect, especially in males. (B) A post-fasting hyperphagia is observed in the 3xtg-AD positive mice when absolute food intake is measured; sgp130Fc clearly decreased this response in males, and also in females, although the effect was minimized. Similar results were obtained when food intake is normalized per body weight. (C) Higher body temperatures were observed in the earlier hours of the day, when 3xTg-AD positive mice showed an increase compared with 3xTg-AD negative. This effect was maintained throughout the day in males. No effect of the inhibition of the IL-6 trans-signaling was observed. Results are MEAN ± SEM. ★ $p \leq 0.05$ vs. 3xTg-AD negative; ▲ $p \leq 0.05$ vs. sgp130Fc negative; ◆ Indicates a significant interaction between both genotypes.

3xTg-AD positive mice showed lower hormonal and lipidic levels in serum, although there were no differences in glycaemia.

Fig. 21A shows the hormones and blood metabolites after the HFD in all genotypes and both sexes. 3xTg-AD positive animals presented lower cholesterol, whereas triglycerides and glucose were normal; no effect of IL-6 trans-signaling was observed in these parameters. In addition, there was a general trend for 3xTg-AD positive animals having decreased levels of insulin, leptin and FGF-21. In males, blocking of IL-6 trans-signaling tended to reverse these AD-elicited changes; in females, in contrast, it tended to further decrease them regardless of the AD genotype.

For analyses of gene expression, a RT-qPCR was carried out in the hypothalamus to check genes related to appetite or metabolism and neuroinflammation (Fig. 21B). No *Agrp*, *Gfap* and *Mac1* differences were obtained between the different genotypes. *Il-6* levels were increased in 3xTg-AD positive males; an effect reversed by the inhibition of the IL-6 trans-signaling as revealed by the significant interaction between both genotypes. Although not significant, the same trend was observed in females.
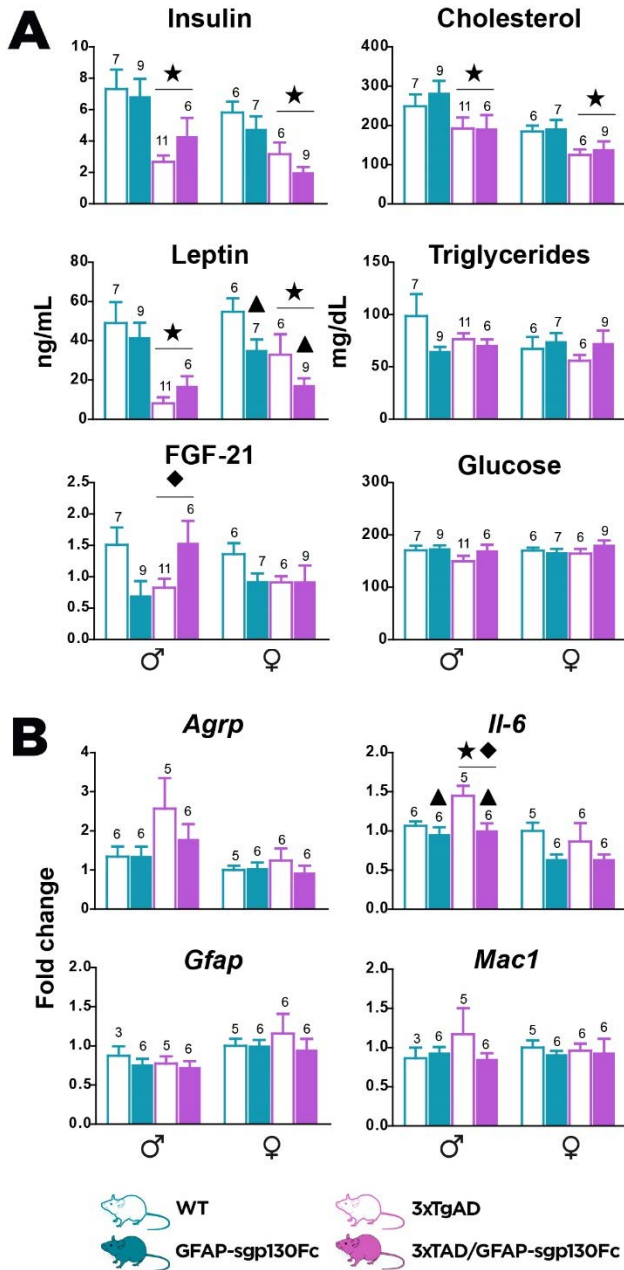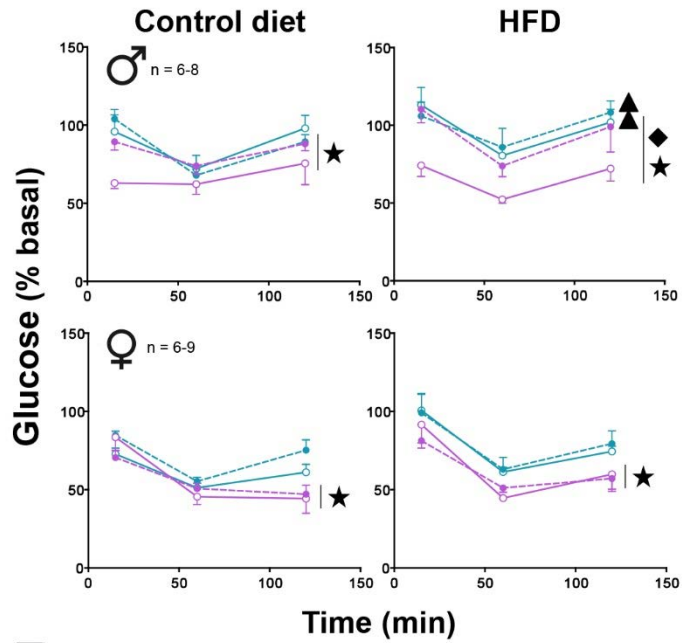
Figure 21. Hormones and metabolites in serum and hypothalamic neuropeptides and inflammatory markers after a HFD exposure. (A) AD transgenes decreased levels of insulin, leptin and cholesterol. The presence of sgp130Fc decreased leptin levels in females and increased Fgf-21 levels in 3xTg-AD positive males. (B) IL-6 mRNA levels were increased in 3xTg-AD positive males; this effect was rescued by the presence of sgp130Fc. Results are MEAN ± SEM. ★ *p≤ 0.05 vs. 3xTg-AD negative;* ▲ *p≤ 0.05 vs. sgp130Fc negative;* ◆ *Indicates a significant interaction between both genotypes.*

3xTg-AD positive mice had hypersensitivity to insulin and increased tolerance to glucose measured by ITT and OGTT, respectively.

Insulin sensitivity and glucose tolerance during both the control and HFD phases are shown in Fig. 22A and 22B, respectively. Insulin sensitivity was increased in 3xTg-AD positive mice in both sexes, and although blocking of IL-6 trans-signaling tended to reverse this increased sensitivity in male mice, it did not reach statistical significance. During HFD sensitivity was still higher in 3xTg-AD positive mice, but in this case the effect of sgp130Fc significantly reversed it in male mice. No trends were observed in this regard in female mice.

In accordance with their increased insulin sensitivity, glucose tolerance was better in 3xTg-AD positive mice during the control diet (Fig. 22B). During HFD, however, that was not significant in male mice, and somewhat surprisingly, in females the opposite emerged. In females with control diet, the blocking of IL-6 trans-signaling decreased this tolerance mostly in 3xTg-AD negative animals as indicated by the significant interaction between genotypes, whereas during HFD a non-significant trend was instead observed in 3xTg-AD positive animals.

A ITT

Control diet — HFD

♂ n = 6-8

♀ n = 6-9

Glucose (% basal)

Time (min)

B OGTT

Control diet — HFD

♂ n = 6-8

♀ n = 6-9

Glucose (% basal)

Time (min)

WT

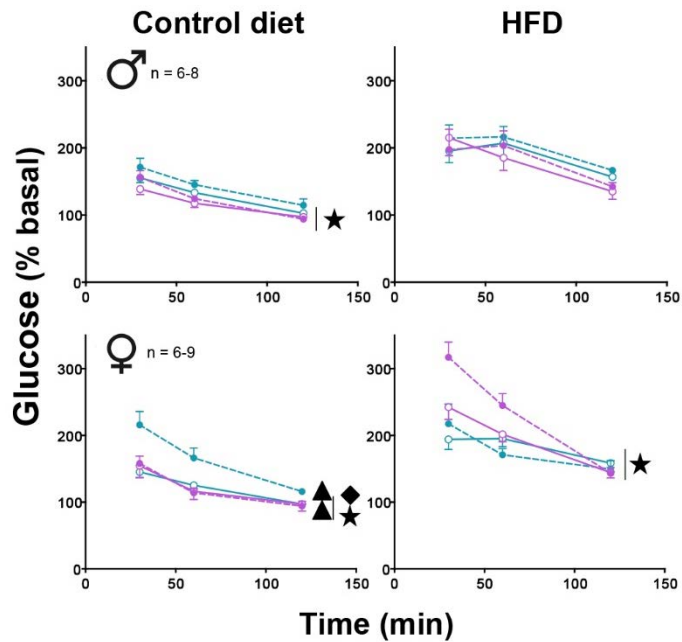GFAP-sgp130Fc

3xTgAD

3xTAD/GFAP-sgp130Fc

Figure 22. Insulin tolerance test (ITT) and oral glucose tolerance test (OGTT) at both experimental phases. (A) None of the genotypes developed insulin resistance with HFD. 3xTg-AD positive animals showed hypersensitivity to insulin in both phases. The inhibition of IL-6 trans-signaling reversed this hypersensitivity in males during the HFD treatment. (B) During the control diet phase, the 3xTg-AD positive mice had better glucose tolerance, in contrast, 3xTg-AD positive females showed the opposite during the HFD treatment. The presence of sgp130Fc increased glucose intolerance in the GFAP-sgp130Fc females with control diet. Results are MEAN ± SEM. ★ $p \leq 0.05$ vs. 3xTg-AD negative; ▲ $p \leq 0.05$ vs. sgp130Fc negative; ♦ Indicates a significant interaction between both genotypes.

AD positive mice had scarce amyloid plaques and reduced Aβ_{40} and Aβ_{42} levels at advanced ages, which were reduced by HFD.

The amyloid plaque load was also evaluated in cortex and hippocampus from females at 20-24 months of age. As expected, amyloid plaques were abundant in the cortex and hippocampus of Tg2576 mice (Fig. 23A top). In contrast, they were very scarce in most of the 3xTg-AD mice (only ~16% of the mice presenting a few plaques in the sections studied), and sgp130Fc did not alter this (Fig. 23A bottom). This precluded attempts of quantification of this immunostaining in a reliable manner.

We next used the more sensitive ELISA to quantify Aβ_{40} and Aβ_{42} levels in cortex and hippocampus from males and females at 20-28 months of age, both from the HFD experiment and from other mice similarly housed fed control diet (Fig. 23B). We noticed that female mice had higher levels than males; that the HFD had in general a decreasing effect; and that the blocking of the IL-6 trans-signaling tended to decrease Aβ_{40} and Aβ_{42} levels in females only in mice fed the control diet.
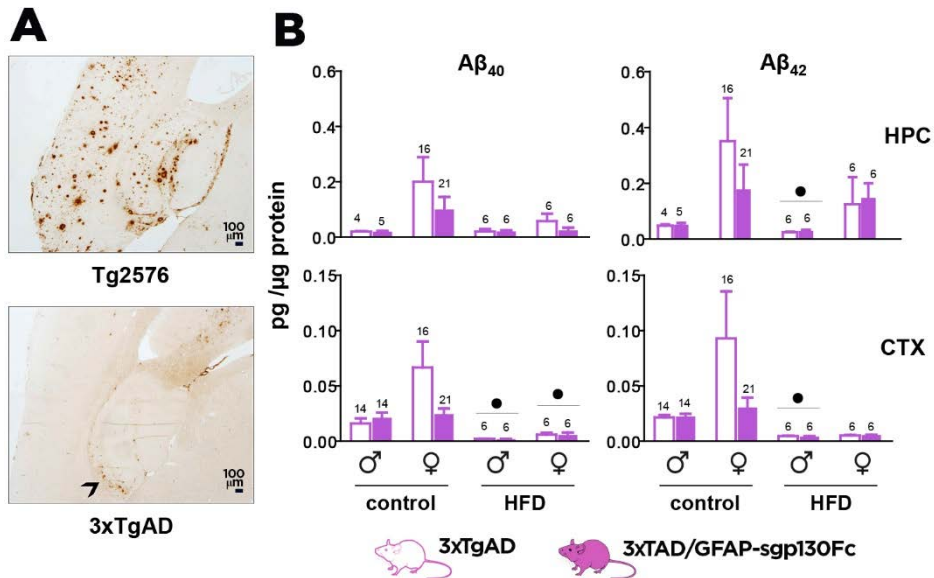
Figure 23. Amyloid plaques detection and Aβ quantification in cortex and hippocampus at advanced ages (20-28 months). (A) Representative Immunostaining from females to analyse amyloid plaque load at 20-24 months of age. Amyloid plaque load was almost undetectable neither in cortex (CTX) nor in hippocampus (HPC). (B) By ELISA, lower levels of $A\beta_{42}$ were detected in cortex and hippocampus from males treated with HFD. The same diet-effect was observed regarding $A\beta_{40}$ levels in males and females cortex. Results are MEAN ± SEM (n=4-21). ● *p ≤ 0.05 vs. control diet.*

3xTg-AD positive female mice with HFD did not show signs of neuroinflammation at advanced ages.

Gliosis in HFD females was analyzed in cortex and hippocampus by GFAP and IBA-1 IHC (Fig. 24A, B). There were no significant effects of AD genes or IL-6 trans-signaling blocking in GFAP immunostaining levels in cortex and hippocampus. This was also the case for the microglial marker IBA-1 in the hippocampus, but, in the cortex blocking IL-6 trans-signaling increased IBA-1 immunostaining regardless of AD genes.
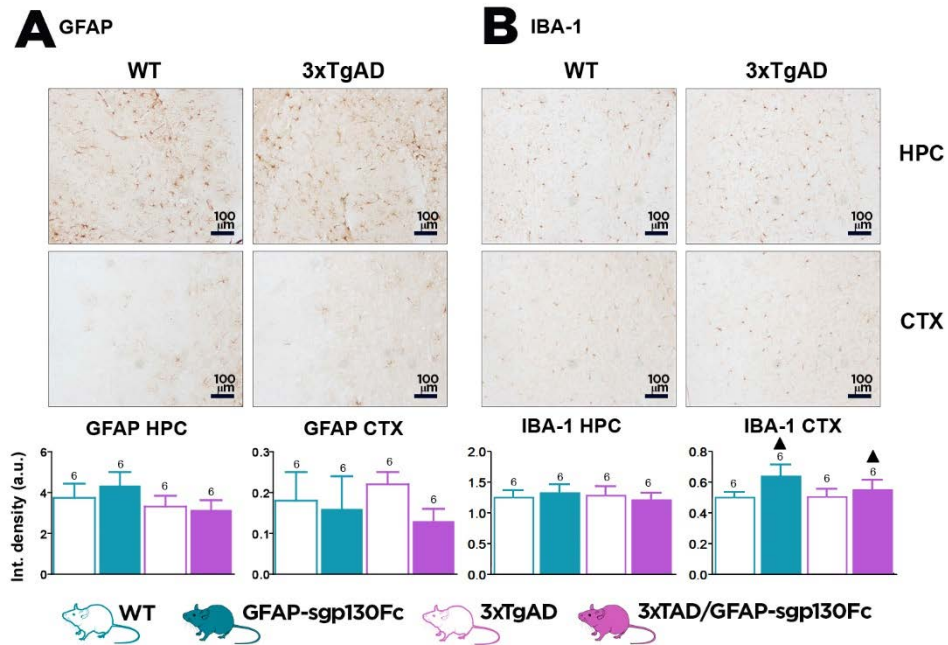
Figure 24. Effects of the inhibition of IL-6 trans-signaling on gliosis in females treated with HFD at 20-24 months of age. (A, B) Representative Immunostaining for GFAP (astrocytes) and IBA-1 (microglia) and the respective quantifications. No effects of the AD transgenes were observed. The inhibition of IL-6 trans-signaling increased IBA-1 staining in cortex (CTX), but not in hippocampus (HPC). Integrated density relativized per area is represented in MEAN ± SEM. ▲ *p≤ 0.05 vs. sgp130Fc negative.*

Discussion

Alzheimer's disease and obesity are major public health problems. They are two of the most prevalent diseases in our society and understanding their pathological mechanisms and the relationship between them will help us to develop successful therapies.

No obvious differences in mortality between genotypes were obtained at younger ages, but when the mice started to become older (>12 months) a trend in having higher mortality in 3xTg-AD positive mice was apparent in both sexes. Decreased survival is a well-known phenomenon in AD mouse models, including the 3xTg-AD (Carlson et al. 1997; Bayer et al. 2003; Manso et al. 2012a, b; Rae and Brown 2015; Torres-Lista et al. 2017; Kane et al. 2018), although the mechanisms involved are poorly understood. Logically, the lifespan of the mice will depend on the presence of the disease and the age-related health deficits (Kane et al. 2018). Furthermore, the higher mortality in 3xTg-AD positive mice is in concordance with their lower body weight. Underweight individuals and weight loss are associated with mortality and morbidity in different neurodegenerative diseases including AD (Aziz et al. 2008). The inhibition of IL-6 trans-signaling in the brain had sex-dependent effects on survival, with apparent detrimental effects in 3xTg-AD positive male mice, and the opposite in females. On the other hand, no effects of either AD or sgp130Fc on early survival were apparent (data not shown). Results with systemic IL-6 KO mice showed normal mendelian patterns (Kopf et al. 1994; Dalrymple et al. 1995), but, when causing IL-6 deficiency in the brain only, a prosurvival role of IL-6 emerged (Quintana et al. 2013; Erta et al. 2015).

Regarding behavior and cognition, the phenotypes of AD- and sgp130Fc-expressing mice were rather subtle, possibly due to the mild disease progression, and were clearly sex-dependent in some cases. At mid ages (~10-11 months), 3xTg-AD positive females showed a trend for decreased horizontal activity (significant during the first minute) and reduced vertical activity (rearings) in the OF, suggesting reduced interest for novelty and/or an increment in emotionality for being exposed to an open and illuminated field, which would be in agreement with previous findings in this mouse model of AD (Giménez-Llort et al. 2007; Sterniczuk et al. 2010). This enhanced emotionality was supported by the increment in defecation in the 3xTg-AD positive mice in every task they were exposed in comparison with the 3xTg-AD negative

animals (Giménez-Llort et al. 2007; Sterniczuk et al. 2010). In the HB, horizontal activity was again essentially normal, and this was also the case when activities in OF and HB were combined. In contrast, an AD-dependent increment in the exploration of the holes was observed in both sexes. In particular, a shorter latency to explore the hole-board holes has also been described in the same animal model at advanced stages of the disease (Giménez-Llort et al. 2007; Sterniczuk et al. 2010; Baeta-Corral et al. 2018) and this is in line with the results in the Tg2576 animal model (Manso et al. 2012a, b). Blocking of IL-6 trans-signaling did not alter horizontal activity and exploratory behaviors in OF and HB, although an increasing trend was observed for the former in OF, significant when considering the ratio between both tests. These latter results might be explained by the stress reduction due to the previous exposure to a similar environment, since both fields are similar in shape. This is in contrast with the decreased activity in systemic IL-6 KO mice (Armario et al. 1998) or deficient in astrocytic IL-6 or IL-6R (Quintana et al. 2013; Erta et al. 2015); the two latter also showed different trends depending on genetic background, age and sex in exploratory behavior. With regard to anxiety, previous studies showed increased anxiety in 3xTg-AD animals compared to controls (Sterniczuk et al. 2010), although in this study an increment in females' open arms entries in the EPM was observed, suggesting lower anxiety levels. Nevertheless, the most specific measure of anxiety, i.e. the percentage of time spent in the open arms, did not reach statistical significance. Therefore, it is likely that the increased exploration (also observed in the HB) and the increased visiting of open arms could be due to a general disinhibition, also reported in some AD patients (Lalonde et al. 2003). It has been previously described that the inhibition of IL-6 trans-signaling in the periphery (using sgp130Fc under the PEPCK promoter) had no effect on anxiety (Braun et al. 2013). In this study we found that the presence of sgp130Fc in the CNS reduced anxiety in males, and the opposite trend was observed in female mice. Interestingly, this is the same pattern observed in mice deficient in astrocytic IL-6 (Quintana et al. 2013; Erta et al. 2015). Because the amyloid and neurofibrillary pathology is predominantly confined, among other areas, to the hippocampus (Billings et al. 2005), MWM was carried out as a measure of hippocampal-dependent spatial navigation and reference memory (Vorhees and Williams 2006). In previous studies it has been shown that 3xTg-AD presented cognitive decline (Billings et al. 2005; Huber et al. 2018). However, AD positive mice showed reduced latencies to find the platform, possibly related to the increment in swim speed in these animals as shown in the supplementary data, which has also been previously observed (Cañete et al. 2015). Blocking IL-6 trans-signaling increased the

latency to reach the hidden platform regardless of AD genes in female mice, which is similar to results found in mice deficient in astrocytic IL-6 or IL-6R (Quintana et al. 2013; Erta et al. 2015), suggesting a decreased spatial learning with either partial IL-6 deficiency or decreased IL-6 trans-signaling. However, these reduced latencies were not consistently reflected in the consecutive probe trials, so there is no real evidence to conclude differences between genotypes. Altogether, these results suggest that the reduction of IL-6 trans-signaling in the CNS does not simply mimic the behavioral phenotype of a reduction of brain IL-6 levels.

3xTg-AD positive mice showed lower body weight than 3xTg-AD negative mice both when fed a normal diet and a HFD, which is similar to the loss of weight present in AD patients (Gillette-Guyonnet et al. 2000). This lower body weight was accompanied by a diminished mass of the white and brown adipose tissues and liver; circulating levels of cholesterol; insulin, leptin and FGF-21 serum levels, hormones that are expected to be regulated according to body weight and fat and/or liver mass; and with the increased insulin sensitivity in the ITT and glucose tolerance in the OGTT (Belgardt and Brüning 2010; Sadagurski et al. 2010; Fisher and Maratos-Flier 2016). Contrary to expectations, 3xTg-AD positive mice had a higher food intake during both phases, both absolute and normalizing by body weight. This increased food intake, despite lower body weight, could be the consequence of the endocrine changes observed, since leptin and insulin inhibit food intake by acting in hypothalamic nuclei. We challenged the mice in the control diet phase with an overnight fasting, and observed that the AD positive mice lost more weight, but upon refeeding they regained weight faster than AD negative mice, likely because they had an increased drive for food intake. These results strongly suggest that the AD positive mice present a hypermetabolic state, which would reconcile their lower body weight with the higher food intake. Indeed, their body temperature throughout the day was also significantly increased. Interestingly, all these effects were more evident in 3xTg-AD positive male mice than in females. These results are in agreement with previous findings where 3xTg-AD animals presented a hypermetabolic state, the reduction in body weight being accompanied by a significant rise in metabolic rate (Knight et al. 2012).

The blocking of IL-6 trans-signaling in the brain during the control diet phase seemed to only mildly affect body weight and food intake in both 3xTg-AD positive and 3xTg-AD negative mice in normal conditions, although a significant reversal of the food intake (normalized by weight) was observed in 3xTg-AD positive male mice. After being challenged with an overnight fast a clear effect of blocking IL-6 trans-signaling

emerged in 3xTg-AD positive mice, since it significantly blunted the regain of body weight upon refeeding, very likely because drive for food intake was reduced, particularly in male mice. In the phase of HFD, the blocking of IL-6 trans-signaling did not influence consistently body weight (i.e. a trend to decrease it was observed in 3xTg-AD negative mice, and the opposite in 3xTg-AD positive mice) or food intake of male mice, but in female mice it decreased HFD-induced obesity, regardless of AD genes. This sex-dependent effect of sgp130Fc was also evident in fat masses, since it tended to reverse the phenotype of 3xTg-AD positive male mice, and to aggravate those of female mice regardless of AD genes. The changes observed in insulin, leptin and FGF-21 levels led to similar conclusions, presumably because these endocrine factors are linked to the changes in body weight, fat depots and liver. In addition, sgp130Fc returned to normal insulin sensitivity in the ITT in 3xTg-AD positive male but not female mice, a trend that was already seen in the control diet phase. Somewhat surprisingly, in female mice sgp130Fc presence in the brain caused poor control of glycemia in the OGTT, but only in either 3xTg-AD negative or 3xTg-AD positive animals in the control diet or HFD phase, respectively. Kraakman and colleagues recently described that mice with peripheral IL-6 trans-signaling inhibition had an increase in body weight, fat mass and leptin levels in serum, although the presence of sgp130Fc prevents (HFD)-induced adipose tissue macrophages accumulation (Kraakman et al. 2015). The study, however, does not indicate the sex of the mice used; and in our experiment, AD positive males did show a trend to increase the body weight gain and fat masses in response to HFD, whereas in female mice the difference is significant. Nevertheless, these putative discrepancies underline the fundamental difference of blockade of IL-6 trans-signaling in the CNS and the periphery. It is also important to emphasize that obesity does increase trans-signaling in the CNS and that it is involved in the metabolic effects of IL-6 by acting in the hypothalamus (Timper et al. 2017). A role of IL-6 in the hypothalamic systems controlling food intake and energy expenditure has been suggested extensively (Benrick et al. 2009; Schéle et al. 2012, 2013; Fernández-Gayol et al. 2019). In our experiment, however, the expression of *Agrp* was not significantly altered in the situations studied.

Somewhat unexpectedly, in this study 3xTg-AD positive animals presented a mild form of the AD neuropathology. Amyloid plaques were really scarce and, amyloid cascade difficult to see by western blot analysis. Soluble $A\beta_{40}$ and $A\beta_{42}$ levels were below the limit of sensitivity of the ELISA at both 5 and 10 months of age (data not shown), but could be detected, yet at low levels (~an order of magnitude below those

of Tg2576 mice (Manso et al. 2012a, b)) at more advanced ages (20-28 months), in both cortex and hippocampus. Also, at least during HFD, no clear signs of glial activation were observed in 3xTg-AD positive mice. This mild phenotype may have to do with (i) the fact that we used hemizygous mice because of the experimental design (need to cross the AD and GFAP-sgp130Fc strains), which shall decrease significantly Aβ production (Oddo et al. 2003a, b; Billings et al. 2005; Hirata-Fukae et al. 2008; Mastrangelo and Bowers 2008; Belfiore et al. 2018); (ii) genetic background of the used strains (Keane et al. 2011); (iii) differences in the housing conditions or in the used methodology (Pasquarelli et al. 2017); and, as our results suggest, the type of diet may also be a factor to consider. However, previous studies do not support an inhibitory effect of HFD in 3xTg-AD females once the pathology is already present (Knight et al. 2014; Christensen and Pike 2017); the same happens in the Tg2576 model (Elhaik Goldman et al. 2018). The putative reasons for these discrepancies are unclear: type of HFD, differences in age when starting the diet, type of housing and specific manipulations done to the animals exposed to HFD might be relevant. Regarding the role of IL-6 trans-signaling, previous studies demonstrated decreased neuroinflammation, including IL-6 production, in response to peripheral LPS if sgp130 is administered icv (Burton et al. 2013); and using the same GFAP-sgp130Fc, a prominent dowregulation of the neuroinflammation caused by the transgenic expression of IL-6 was observed (Campbell et al. 2014). In agreement with that view, in the hypothalamus of 3xTg-AD positive male mice *Il6* gene expression was increased significantly, and this was blocked by sgp130Fc. But in the cortex and hippocampus, the main areas where amyloidosis is to be expected, GFAP was unaffected, whereas IBA-1 immunostaining was in fact increased regardless of AD genes, by transgenic sgp130Fc. Whether this is a consequence of the mild phenotype of the 3xTg-AD groups, or if this would occur during control diet is unclear. Yet, $A\beta_{40}$ and $A\beta_{42}$ levels tended to be decreased by the blocking of IL-6 trans-signaling in female mice with control diet, which is in line with the expected downregulation of neuroinflammation by sgp130Fc in this model.

Conclusions

A complex study to shed light to the understanding of AD and obesity has been carried out. The mouse model, 3xTgAD, has shown a mild amyloid phenotype in our

experimental conditions. Yet, significant effects were observed in survival, some behavioral traits and learning, and in the control of body weight with control and HFD, and related endocrine and metabolic factors. The inhibition of IL-6 trans-signaling modulated the AD phenotype, and also that of AD negative mice, in a sex-dependent manner. Further work with homozygous 3xTg-AD mice, as well as in other mouse AD models, is warranted to better understand the role of IL-6 in general and of IL-6 trans-signaling in particular, in this regard.

GENERAL DISCUSSION

Alzheimer's disease (AD) is one of the most prevalent causes of dementia in elderly people (Ittner and Götz 2011). It courses with neuroinflammation, including secretion of pro-inflammatory cytokines such as IL-6 (Bertram et al. 2010). Many of the neuropathogenic effects of IL-6 in the CNS are mediated via trans-signaling, and its inhibition using the sgp130Fc protein reversed some of the detrimental effects in GFAP-IL6 mice (Campbell et al. 2014). For the aforementioned evidences, we decided to characterize the progression of the pathology in two widespread AD mouse models, Tg2576 and 3xTg-AD, when the IL-6 trans-signaling is inhibited in the CNS.

Both AD models have been discussed in their respective chapters. Herewith, we will focus in the comparison between them.

Amyloidosis

As mentioned before, Aβ can be presented as monomers, oligomers (dimers, trimers, etc.) and fibrils. Some studies described that Aβ oligomers correlate better with memory dysfunction than amyloid plaque deposits (Lesné et al. 2006, 2013). Hence, it is worthy to measure amyloidosis using different approaches. In the present study, Western blot and ELISA were used to evaluate the amyloid cascade, including full length APP, CTF-β fragment and monomeric Aβ. Besides, immunohistochemistry was used to measure amyloid plaque load and deposition.

Previous studies described that the Tg2576 mice present a progressive increment in Aβ concentration, developing amyloid plaques at approximately 12 months of age (Hsiao et al. 1996; Manso et al. 2012a). In contrast, 3xTg-AD mice are characterized by a faster amyloid pathology development. In homozygous mice, extracellular plaques are detected at 6 months; in heterozygous, the amyloid pathology is delayed, but both display numerous extracellular deposits by 15 month of age (Oddo et al. 2003a, b).

Regarding old Tg2576 mice, the results of amyloidosis obtained in IHC are in line with the previously described, and numerous amyloid plaques were found in cortex and

hippocampus. In contrast, 3xTg-AD mice started to develop amyloid pathology later than expected as amyloid plaques were really scarce and detected only in 16 % of the subjects after 20 months of age. As mentioned in the Chapter 2, the exact reason for the mild phenotype is unclear, however a genetic drift has also been described by other authors (Marchese et al. 2014; Caccamo et al. 2015; Wu et al. 2015; Jankowsky and Zheng 2017).

The presence of sgp130Fc reduced amyloid plaque load in the Tg2576 females in both areas. No changes were observed in Tg2576 males. In case of the 3xTg-AD model, it has been difficult to assess the effect of the inhibition of the IL-6 trans-signaling in amyloid deposition as a consequence of the very mild AD phenotype. Nevertheless, it was possible to measure monomeric $A\beta_{40}$ and $A\beta_{42}$ levels using ELISA in both AD models. As expected, Tg2576 mice displayed higher amount of $A\beta$ in cortex and hippocampus in comparison with 3xTg-AD mice. Tg2576/GFAP-sgp130 females had reduced $A\beta_{40}$ and $A\beta_{42}$ levels in cortex in comparison with the Tg2576 females; the same effect was observed in WB from cortex, although not significantly. In contrast, WB from females' hippocampus revealed an increase of APP and APP-derived fragments in the double transgenic animals, which was not observed in the ELISA from this tissue. The reason for the incompatibility in the detection of monomers between techniques is unknown; however, as brain samples were homogenated using SDS and then loaded to SDS-page, a disaggregation of the oligomeric $A\beta$ and accumulation at the end of the gel should not be discarded. The influence of sgp130Fc in APP processing and deposition might be different between both areas, in cortex it inhibited the formation of monomers and plaques, whereas in hippocampus it promoted APP and its proteolityc fragments, but inhibiting amyloid deposition. A correlation between the different $A\beta$ pools has been described previously, the first step of fibril assembly involves the formation of oligomers, which can further associate to form amyloid fibrils or not (Iadanza et al. 2018; Zhang et al. 2018). Some authors stated, that amyloid growth occurs by the addition of monomers in a independent and competitive reaction to the formation of toxic oligomeric species (Collins et al. 2004); hence, the formation of amyloid plaques could represent a protective mechanism against the formation of toxic $A\beta$ oligomers (Lee et al. 2005). In the 3xTg-AD model, a trend to have reduced $A\beta$ levels was observed in cortex and hippocampus of female mice with blocked IL-6 trans-signaling. Again, no effects of the inhibition of IL-6 trans-signaling were observed in males.

Altogether, these results hint a possible role of IL-6 trans-signaling in Aβ production and deposition. The sgp130Fc reduced both soluble $A\beta_{40}$ and $A\beta_{42}$ levels (at least in cortex) and Aβ deposition in cortex and hippocampus, although only in females. Generally, females from both AD models showed more pronounced amyloid pathology; this effect was previously observed and mimics the higher AD prevalence in women; the reason is still unclear, even though it could be attributed to effects of estrogens in Aβ processing (Callahan et al. 2001; Allué et al. 2016).

As stated in Chapter 1, how IL-6 trans-signaling interferes in amyloid pathology is still unclear, however, possible explanations such as deficits in matrix organization and vascular alterations have been pointed out in the Chapter. The only known proteases to cleave off N- and C-terminal pro-domains of the matrix proteins collagen types I and III are meprin α and β (Arnold et al. 2017b), which besides also cleave IL-6R what will increase the soluble form and, in turn, IL-6 trans-signaling (Arnold et al. 2017a). In addition, meprin β cleaves APP to produce truncated non-toxic N-APP fragments (Jefferson et al. 2011) but also Aβ (Bien et al. 2012). The importance of meprin β in AD has to be elucidated, but, an interplay between these factors (meprins, collagen, IL-6 trans-signaling and Aβ) could have a role in the disease progression and should be studied in detail.

Usually, AD is accompanied by pronounced neuroinflammation and the release of pro-inflammatory cytokines. Also, an increment in gliosis, with marked astroglia and microglia activation especially surrounding amyloid plaques, as well as secretion of pro-inflammatory cytokines including IL-6 has been seen in both AD models previously (Frautschy et al. 1998; Oddo et al. 2003a; Chakrabarty et al. 2010; Fuhrmann et al. 2010; Comes et al. 2017). Tg2576 mice bred in our lab from both sexes showed enhanced gliosis; marked GFAP and IBA-1 staining surrounding amyloid plaques was detected in cortex and hippocampus from females, however, the gliosis in males was more subtle, as only enhanced GFAP staining was observed in cortex. An enhanced expression of *Gfap* was also observed in the microarray when APP factor was analyzed. Additionally, when the presence of sgp130Fc was measured in the cortex from these mice, a trend to be increased in the APP-positive animals from both sexes was showed which is directly linked with higher GFAP expression. Some what surprisingly, the reduction in plaques did not correlate with a reduction in either astrogliosis or microgliosis as measured by IHC, as well as it was not observed in WB (data not shown). Nevertheless, this is in agreement with the microarray analysis, as no inflammatory markers were differentially expressed in the analysis of the

interaction between factors. In the 3xTg-AD model no enhanced gliosis was observed in the 3xTg-AD positive mice in comparison with the 3xTg-AD negative controls using IHC, probably due to the lack of amyloid plaques. In line, no increased mRNA of GFAP or IBA-1 in the hypothalamus was showed, although an increase in IL-6 mRNA was observed in the AD positive mice. The latest effect was reversed by the inhibition of IL-6 trans-signaling, which is in line with previous results in aged mice (Burton et al. 2013).

Survival

The comparison in terms of survival between different AD models is difficult. Some studies have demonstrated different life expectancy among inbred mouse strains and among different mouse models of AD, and besides, sex differences had been observed within the same strain or AD model (Krezowski et al. 2004; Kane et al. 2018). Additionally, differences between laboratories in housing conditions, breeding experience, exercise, stress levels or diet can influence survival (Rae and Brown 2015). Particularly, in the Tg2576 model big differences between labs have been reported ranging from a median of 68 days for males and 80 days for females (Westmark et al. 2008) to 189 days for males and over 350 days for females (Freude et al. 2009). In contrast, the median lifespan reported in 3xTg-AD mice was 469 days in males and 744 days in females (Rae and Brown 2015).

All these differences highlight the need for a common scale of aging between models; the definition of common age ranges for mature adults, middle aged and old mice would help us to define better disease stages and would allow the comparison between different AD models. In fact, it is important to make a distinction between longevity (lifespan) and healthy aging (healthspan) or the length of time that an individual is able to maintain good health. The most common measure of healthy aging is the "Frailty Index (FI)" which counts the number of health-related deficits a person or mouse has accumulated divided by the number of deficits assessed, resulting in a FI score between 0 and 1 (Whitehead et al. 2014; Kane et al. 2018). The FI index correlates with mortality and amyloid-beta plaque load (Wallace et al. 2013; Kane et al. 2018), hence, it could be a good choice in order to compare survival and neuropathological deficits in the different AD mouse models and facilitate the comparison, after all, with humans.

Tg2576 females showed higher early mortality (before weaning) than AD negative controls. This effect was also observed in previous studies, which suggested an

important role of the APP family members in embryonic and early postnatal development (von Koch et al. 1997; Heber et al. 2000; Phinney et al. 2003; Manso et al. 2012b, 2016). It was not possible to assess this APP lethal effect in the 3xTg-AD model as they and their respective AD negative controls were not littermates (born from different crossbreedings).

The inhibition of IL-6 trans-signaling did not affect the mendelian ratios in any of the two models, despite this cytokine is as expressed early as the eight-cell stage embryo (Zolti et al. 1991). These results are in line with previous using the systemic IL-6 KO mice (Kopf et al. 1994; Dalrymple et al. 1995), although a reduction in early survival was observed in astrocyte-IL6 KO and astrocyte-IL6R KO mice (Quintana et al. 2013).

A reduced lifespan was also showed by both AD models in comparison to their respective AD negative controls. Tg2576 mice suffered a drastic mortality, starting at approximately 4 months (at 16 month of age the mortality was approximately 50%). In contrast, 3xTg-AD mice showed this phenotype later in life, at 24 months of age. The inhibition of IL-6 trans-signaling tended to rescue this phenotype in Tg2576 mice from both sexes, although only significantly in females. Whereas the same effect was observed in 3xTg-AD females, the opposite was observed in males. As stated in the previous chapters, the lifespan of the AD mice will depend on the evolution of the disease and the age-related health deficits (Kane et al. 2018). There is a correlation between amyloid cascade and/or amyloid plaques deposition and mortality. Firstly, increased Aβ clearance by transgenic synthesis of Aβ degrading enzymes (IDE or NEP) prevents plaque formation and premature death in APP overexpressing mice (Swedish and Indiana mutations) (Leissring et al. 2003). And secondly, the deletion of Ccr2 impairs microglial recruitment and this favors early Aβ accumulation and increases premature death in Tg2576 mice (El Khoury et al. 2007). However, it seems likely that the premature death involves more mechanisms than APP-induced death (Krezowski et al. 2004).

It is difficult to define a specific cause of mortality as mice will die of a variety and sometimes unknown causes (Rae and Brown 2015). Nevertheless, in Chapter 1, seizures were highlighted as one of the main causes of premature mortality in the Tg2576 model (Westmark et al. 2008). IL-6, particularly IL-6 trans-signaling has a role in the modulation of basal synaptic transmission in different ways. Some studies demonstrated that IL-6 KO mice have increased seizures susceptibility to some convulsant stimuli (i.e. pentylenetetrazole (PTZ), kainic acid or NMDA (Penkowa et

al. 2001; De Sarro et al. 2004) or audiogenic stimuli (De Luca et al. 2004). In line, GFAP-sgp130Fc were also more sensitive to PTZ-induced seizures (Cuevas-Olguin et al. 2017). Interestingly, GFAP-IL6 mice also presented a severe neurologic disease which includes seizures (Campbell et al. 1993) and higher susceptibility to kainic acid- and NMDA-induced seizures (Samland et al. 2003). These evidences suggest a complex system with dual effects of IL-6. Our results indicated a reduced mortality in Tg2576/GFAP-sgp130Fc mice; if it is as a consequence of a reduction in seizures remain uncertain as no electrophysiological measures were studied. We did not notice convulsions during the day; can not rule out they happened overnight. In the 3xTg-AD mice, enhanced premature mortality has been attributed to an immune system dysfunction and severe splenomegaly (Giménez-Llort et al. 2008). Splenomegaly was also observed in our 3xTg-AD male mice; the blocking of IL-6 trans-signaling diminished this effect (data not shown). However, it is not simply correlated with mortality since it was higher in the 3xTg-AD/GFAP-sgp130Fc males in comparison to the 3xTg-AD group.

Vascular dysfunctions have also been proposed as a cause of premature death. Tg2576 mice display an enhanced cerebral amyloid angiopathy (CAA) at 9-12 months of age (Klohs et al. 2014), reduced cerebral blood flow (CBF) (Niwa et al. 2002b) and impaired cerebrovascular autoregulation (Niwa et al. 2002a). Moreover, Tg2576 show clear signs of oxidative stress, which may cause endothelial damage. Overexpression of human superoxide dismutase-1 (SOD1) decreases this damage and also premature death, supporting a role of oxidative stress/neuroinflammation in the premature mortality (Carlson et al. 1997). In accordance, our microarray results showed that numerous genes involved in the organization of collagen fibrils and the development of blood vessels were upregulated by blocking IL-6 trans-signaling in the hippocampus of Tg2576 females. In line, GFAP-IL6 mice presented an increment in laminin deposition and a marked dilatation of blood vessels in the cerebellum, besides an increased BBB leakage; these disturbances were reduced by the inhibition of the IL-6 trans-signaling in the CNS using the sgp130Fc protein (Campbell et al. 2014). Additionally, the inhibition of IL-6 trans-signaling, using also the sgp130Fc protein, prevented inflammation and endothelial barrier disruption in retinal endothelial cells when exposed to IL-6 and sIL-6 (Valle et al. 2019).

Overall, our results suggested that the inhibition of IL-6 trans-signaling improves AD premature mortality, especially in Tg2576 female mice. Although the reason is

unclear, we suggest, based in our results, that an improvement in the vascular system of the double transgenic mice could be a feasible explanation.

Behavior

Behavior has been assessed in both AD animal models at different time points. Tg2576 animals have been studied before (~4-6 months) and after the amyloid plaques deposition (~14-18 months), whereas, 3xTg-AD mice were studied at young (~5-6 months) and mid ages (~10-11 months), both before amyloid plaques deposition. Different paradigms have been used to characterize different facets of their behavior as locomotion, exploration, anxiety and spatial memory and learning.

Locomotion, emotionality, exploration and anxiety

When mice are exposed to a novel/unknown environment, they experience different situations (i.e. curiosity, fear, anxiety). The behavior of the animals is multidimensional, so, different complementary tests have been designed in order to measure these behaviors.

Open field (OF) allows us to test general locomotor activity, exploration in a novel environment and some anxiety-related behaviours in rodents (Walsh and Cummins 1976). Some researchers have interpreted high locomotion (horizontal activity) or exploration (vertical activity, rearings) in this test as an index of low emotionality, although some others conceive them as independent parameters (Denenberg 1969; Seibenhener and Wooten 2015). Although its validity is controversial, defecations in the OF have also been used as a measure of emotionality as highly emotional animals exhibit increased defecations boli; indeed, locomotion and defecations are usually negatively correlated (Archer 1973). Anxiety can also be measured in the OF, since being close to the walls when exploring an open space (thigmotaxis) has been correlated with increased anxiety (Seibenhener and Wooten 2015). The hole-board (HB) is better than OF to evaluate exploration, as head-dipping into holes in the floor is assumed to be a valid measure of the subject's attraction towards novelty (Brown and Nemes 2008). Finally, the elevated-plus maze (EPM) is commonly used to measure anxiety; an increase in open arm activity (duration and/or entries) reflects a decrease in the anxiety behavior (Pellow et al. 1985; Walf and Frye 2007). Collecting information from the different tests allows us to have a broader view of the psychobiology of these mice.

It is generally accepted that Tg2576 mice present hyperactivity and reduced anxious behavior, despite some inconsistencies have been found in the bibliography due to either extrinsic (lab conditions, diet or manipulation), or intrinsic (genetic backgrounds, age and sex) causes (Chapman et al. 1999; King and Arendash 2002; Lalonde et al. 2003; Ognibene et al. 2005; Deacon et al. 2008; Lassalle et al. 2008; Toda et al. 2011; Manso et al. 2012b, a, 2016). Young females (~4-6 months) presented increased activity and exploration measured in the OF and HB, respectively, and reduced anxiety in the EPM. The latest is attributed to an increase in disinhibitory tendencies which were previously reported in Tg2576 mice compared to WT (Lalonde et al. 2003; Ognibene et al. 2005). Some authors attribute the disinhibition to a disruption in the normal cholinergic function (Ognibene et al. 2005), which is likely to happen in the Tg2576 mouse model, although contradictory results have been found (Lüth et al. 2003; Wenk et al. 2004; Watanabe et al. 2009). The disinhibition is also characteristic in some AD patients (Lalonde et al. 2003). The APP-dependent effects were less marked at ~4-6 months in males, since only an increment in open arms entries was observed. Nevertheless, an increased number of defecations, and thus increased emotionality, was observed in these young males. After the amyloid plaques deposition, APP positive females still presented hyperactivity, increased exploration and reduced anxiety. APP positive males also showed increased activity and reduced anxiety, but not increased exploration or emotionality, suggesting a certain degree of habituation to the test or to manipulation.

As no 3xTg-AD negative animals were available at 5-6 months, it was not possible to test the 3xTg-AD effects at this age. At ~10-11 months, 3xTg-AD mice presented a more subtle behavioral profile than the Tg2576. Yet, increased exploration was observed in the HB, which is partially in agreement with previous observations (Baeta-Corral et al. 2018), but different from others (Giménez-Llort et al. 2007; Sterniczuk et al. 2010). In contrast to the enhanced exploration in the HB, a decrease in vertical activity (rearings) and horizontal activity in the first minute was observed in the OF. This reduced exploration, in addition to the increment in defecations boli in all test, suggest an elevated emotionality, which was previously reported (Giménez-Llort et al. 2007). The contradictory results in exploration between tests could be because of habituation to the test cage as both are similar in shape and color. Regarding anxiety, a trend in reduced anxiety was observed in females, although only is significant in number of entries to open arms. This is not in agreement with previous studies, which

described increased anxiety in the EPM (Sterniczuk et al. 2010; Baeta-Corral et al. 2018).

The inhibition of IL-6 trans-signaling had effects in both AD models, although more pronounced in the Tg2576, possibly due to the mild disease condition of the 3xTg-AD and consequently the poor inflammation. In the Tg2576 mice, the blocking of the IL-6 trans-signaling partially reversed the APP-dependent effects on exploration and anxiety (but not activity) of young females. Interestingly, the blocking of IL-6 trans-signaling was no longer effective in old females regarding exploration and anxiety. In old males, the inhibition of IL-6 trans-signaling reversed the now present hyperactivity. In old females, however, the opposite was observed. Regarding the 3xTg-AD mice, the sgp130Fc protein only had effects on anxiety at 10-11 months, decreasing it in males independently from the AD transgenes. The implication of IL-6 in modulating many behavioral traits has already been reported; systemic IL-6 KO (Armario et al. 1998) and astrocytic-IL6 KO (Quintana et al. 2013; Erta et al. 2015) mice presented hipoactivity in the HB, which is in contrast to the increased motility obtained in the OF by Butterweck and colleagues (Butterweck et al. 2003). Regarding exploration, IL-6 KO mice showed reduced exploratory behavior measured by the NORM (Baier et al. 2009). In agreement, a sex-dependent reduction has been observed in astrocytic-IL-6 or astrocytic-IL6R KO mice using the HB (Quintana et al. 2013; Erta et al. 2015). Previous results regarding anxiety are not that clear. The systemic KO mice presented low open arms exploration (Armario et al. 1998) and decreased rearings in the OF (Butterweck et al. 2003), suggesting higher anxiety and hence an anxiolytic role of IL-6; in line, astrocytic IL-6 KO mice spent less time in open arms at 8 months, however, a subtle opposite effect was observed in astrocytic-IL6 deficient males at 6 weeks (Quintana et al. 2013; Erta et al. 2015). Few studies have been done in order to unravel the role of IL-6 trans-signaling in behavior, however, Braun and colleagues reported a reduced exploratory behavior in PEPCK-sgp130Fc animals (with the IL-6 trans-signaling blocked in the periphery) using the novel object recognition memory test (NORM), which is in accordance with our results. No effects were observed in anxiety or activity in this case (Braun et al. 2013).

Learning and memory

The MWM was used to assess hippocampus-dependent spatial learning and memory (Billings et al. 2005; Vorhees and Williams 2006). Memory loss is one of the main features of AD and one of the most incapacitating (Burns and Iliffe 2009); hence, the

presence of cognitive impairment is vitally important for AD animal models to represent meaningful models of this devastating disease. As far as we know, most AD mouse models present cognitive deficits, including the Tg2576 (Hsiao et al. 1996) and the 3xTg-AD (Billings et al. 2005). When first characterized by Hsiao and colleagues, Tg2576 mice presented performance deficits in Y-maze and MWM at 9-10 months of age (Hsiao et al. 1996). Although there is not a clear consensus about the age of onset of the memory impairment, many studies are able to detect it (King and Arendash 2002; Lassalle et al. 2008; Manso et al. 2012b, 2016), but not all (Bizon et al. 2007; Zhuo et al. 2007; Manso et al. 2012a). As in humans, some authors highlight the importance of Aβ oligomers on cognitive deficits rather than amyloid plaques, particularly the Aβ*56 oligomer (Aβ dodecamer) seems to be responsible for the first memory deficits at 6 months of age in mice (Lesné et al. 2006; Forloni and Balducci 2018). In fact, the elimination of oligomers using the compound RD2 reversed cognitive deficits and significantly reduced Aβ pathology in old-aged transgenic APP/PS1 mice (Schemmert et al. 2019). Contradictory results have also been found regarding the 3xTg-AD model, as while it is generally accepted that these mice display spatial learning and memory deficits (Knight et al. 2014; Cañete et al. 2015; Torres-Lista et al. 2017), some authors failed to detect them in the MWM using the classical measures (Baeta-Corral et al. 2018), stating that perhaps the MWM may probably not be specific to assess hippocampal-dependent memory in the 3xTg-AD mice, as they display a sustained increase in swimming speed, which reflects anxiogenic effects.

In agreement with the positive correlation between oligomers and memory impairment, our Tg2576 animals showed cognitive deficits before and after the amyloid plaques deposition. At ~4-6 months of age, during the acquisition of the hidden platform phase, only males displayed cognitive deficits, particularly in the first days, when the animals had to cope with a new stressful situation and reduce their noncognitive/emotional responses such as thigmotaxis (Lipp and Wolfer 1998). Nevertheless, both sexes showed deficits in spatial retention in both probe trials, immediately after the last training trial (short-term memory) and 24 hours after (long-term memory). In contrast, during the reversal phase, APP positive males showed the same cognitive flexibility than their respective APP negative controls, whereas, APP positive females showed an impaired learning process, which was evidenced after 24 hours, in the probe trial. After amyloid plaques deposition, APP positive female mice showed a deficient performance in both test phases, acquisition and reversal.

Regarding the 3xTg-AD mice, no defects in cognition were detected at 5-6 or 10-11 months of age. 3xTg-AD positive animals showed an increased swimming speed throughout the test in comparison to 3xTg-AD negative controls, whereas no differences in speed were detected between sgp130Fc positive and negative mice. This increased swimming speed leads to better latencies to achieve the submerged platform in the 3xTg-AD positive mice. However, the total distance moved was the same when compared with 3xTg-AD negative mice, indicating similar strategies of navigation. In agreement, no consistent improvement in short- and long-term memory was observed in the 3xTg-AD positive animals in the probe trials. Taking these measurements into account, there is no real evidence to conclude differences in learning and memory between 3xTg-AD positive and 3xTg-AD negative mice.

The inhibition of IL-6 trans-signaling had modest effects in learning and memory and only in females. In old Tg2576 females, the blocking of the IL-6 trans-signaling improved short-term memory from the acquisition phase. In contrast, it deteriorated that of the reversal phase regardless of age and *App* transgene. Although a reduction in plaques was observed in hippocampus from double transgenic females, only minor improvement was observed in the performance of the MWM. In principle, this fact highlights the importance of oligomeric Aβ in cognition. Regarding the 3xTg-AD model, the double transgenic 3xTg-AD females at 5-6 months presented reduced latencies to find the hidden platform and consequently, they spent significantly more time in the TQ during the immediate probe trial test indicating an improvement in cognition. However, the opposite was observed at 10-11 months, the sgp130Fc positive females (independently from AD transgenes) had higher latencies to reach the platform and increased distance moved in the acquisition, suggesting a deficient spatial learning and strategy of navigation which was translated in a deteriorated long-term memory of the 3xTg-AD/GFAP-sgp130 mice.

Altogether, it seems that the effects of the inhibition of the IL-6 trans-signaling on hippocampus-dependent learning and memory are sex, age, and model-dependent. It had beneficial effects in old Tg2576 females when they had to remember were the platform was located (acquisition), but it was detrimental when the Tg2576 females (old and young) had to re-learn new information suggesting an impaired cognitive flexibility (reversal). Regarding 3xTg-AD females, the inhibition of IL-6 trans-signaling improved the performance in the acquisition at 5-6 months of age, whereas, it had detrimental effects in the same test phase at 10-11 months. No consistent effects were obtained in cognitive flexibity in this case. Controversial results have been

reported previously in the systemic IL-6 KO mice, some authors stating a deficient hippocampus-dependent and independent learning (Baier et al. 2009), whereas others reporting the opposite (Braida et al. 2004). Studies with astrocyte-IL6 and astrocyte-IL6R found out an impaired spatial learning in these mice (Erta et al. 2015). No effects on spatial learning and memory were observed when IL-6 trans-signaling was inhibited in the periphery, using the sgp130Fc under the PECK promoter (Braun et al. 2013). Therefore, only the IL-6 trans-signaling from the CNS has a role in spatial learning and memory, but it does not mimic perfectly the total effects of IL-6 deficiency.

Body weight and metabolism

In this thesis, body weight has been assessed under different circumstances. Firstly, under normal chow control diet from weaning up to euthanasia and, secondly, exposing the animals to 10 weeks of high-fat diet (HFD). Both models carried out the control diet phase, but only 3xTg-AD mice performed the HFD experiment at ~19 month of age. In addition to body weight assessment, more parameters have been measured in the 3xTg-AD mice such as fasting and refeeding, insulin response (ITT and OGTT), body temperature, many hormones, metabolites and neuropeptids and total mass of different tissues.

Tg2576 mice presented lower body weight in comparison with their respective APP negative controls at all ages. Numerous studies have reported it previously (Manso et al. 2012a, b, 2016; Ishii et al. 2014); the reduction in body weight could be attributed to an increase in energy expenditure rather than alterations in feeding behavior. Besides, these mice exhibit early metabolic deficits and a pathologically low leptin state associated with a hypothalamic dysfunction in arcuate neuropeptide Y neurons, hence, they present a dysregulation in the production of orexi/anorexigenic peptides (Ishii et al. 2014). Our 3xTg-AD mice also presented reduced body weight, and besides, increased food intake under normal and fasting conditions, hypersensitivity to insulin and better glucose tolerance than 3xTg-AD negative controls. These results are in accordance with previous studies (Knight et al. 2012; Marchese et al. 2014). The hyperphagia is attributed to a defective gut-brain signaling, with a reduction in the sensitivity to the anorexic actions of certain satiety factors such as cholecystokinin (CKK) (Adebakin et al. 2012). In agreement, an hypermetabolic state in the 3xTg-AD model has been reported previously (Knight et al. 2012, 2014), characterized by hyperphagia, low body weight as previously mentioned and a significant rise in the metabolic rate indicated by higher oxygen consumption and carbon dioxide

production. In line, our 3xTg-AD mice presented higher body temperature than AD negative animals, especially during the first hours in the morning. Although previous studies demonstrated the implication of IL-6 in body weight regulation (i.e. IL6-KO mice develop mature-onset obesity (Wallenius et al. 2002b) whereas GFAP-IL6 mice are resistant to HFD-induced obesity (Hidalgo et al. 2010)), no effects of the inhibition of IL-6 trans-signaling were observed on basal body weight on either of the two AD models. However, when food intake was measured in 3xTg-AD mice significant changes appeared; in normal conditions it reversed the hyperphagia in 3xTg-AD males, whereas, in fasting conditions, the reversion was significant in both sexes, preventing then the body weight recovering upon refeeding. Besides, modest effects of the inhibition of IL-6 trans-signaling were observed in glucose metabolism in these conditions; it only decreased glucose tolerance in the GFAP-sgp130 females. No changes were observed in body temperature produced by the blocking of IL-6 trans-signaling.

In HFD conditions, 3xTg-AD animals also presented reduced body weight, as well as, diminished mass of the white and brown adipose tissues and liver in comparison with the 3xTg-AD negative controls. In line, reduced levels of insulin, leptin and cholesterol were observed, as these factors are regulated accordingly to the fat mass. The hyperphagia is maintained in the 3xTg-AD animals under HFD conditions, likely due to the low anorexic signals produced by insulin and leptin. Additionally, insulin hypersensitivity and better glucose tolerance is also showed by the 3xTg-AD animals in this condition. Only males did not present the latest effect. The majority of IL-6 trans-signaling effects on body weight and metabolism has been studied under HFD conditions: the central administration of IL-6 in mice suppresses feeding behavior and improve glucose tolerance, particularly, IL-6 trans-signaling is enhanced in the CNS of obese mice, intensifying the IL-6 metabolic effects (Timper et al. 2017). Besides, IL-6 trans-signaling from the periphery mediates the macrophage recruitment into adipose tissue (ATM) in a HFD-induced obesity animal model (Kraakman et al. 2015). In the present thesis, a role of IL-6 trans-signaling in body weight regulation and metabolism of 3xTg-AD mice under HFD conditions has been demonstrated. In males, the 3xTg-AD with blocked IL-6 trans-signaling mice showed a trend to have increased body weight in comparison to 3xTg-AD mice; accordingly, they showed increased weight of different adipose tissues and increased levels of insulin and leptin. Fgf-21 levels in serum were also increased in these animals; in line, increased serum Fgf-21 levels in obese state have been previously reported, both in mice (Fisher et al.

2010) and humans (Zhang et al. 2008), suggesting a Fgf-21 resistance in obesity. Besides, it also reduced the insulin hypersensitivity in the double transgenic male mice. However, the opposite was observed in females, the inhibition of the IL-6 trans-signaling reduced total body weight and adipose tissues in both, 3xTg-AD negative and 3xTg-AD animals, enhancing then, the hypermetabolic phenotype; consistently, it decreased leptin levels significantly. Differences in body weight regulation between sexes have been described previously in animals 3xTg-AD animals exposed to HFD (Barron et al. 2013), as well as, in muscle-IL6 KO mice (Ferrer et al. 2014). The reasons for these gender-specific differences still unclear, however, it is very likely to be attributed to hormonal factors (Yakar et al. 2006).

The aforementioned evidences suggested a hypermetabolic state in the 3xTg-AD animals which is modulated, in a sex-dependent manner, by the inhibition of IL-6 trans-signaling. On one hand, in males, no significant effects of the blocking of the IL-6 trans-signaling on body weight or tissues mass were observed, however, a trend to reverse the 3xTg-AD-dependent effects was showed in both parameters, and consequently, on the secreted hormones after HFD. In line, the sgp130Fc reversed the hyperphagia in normal and fasting conditions. On the other hand, in females, the opposite effects were observed. The sgp130Fc potentiated the 3xTg-AD-effects in body weight, tissues mass and hormones. No changes were observed in food intake in normal conditions, in contrast, the same effects than in males were observed after fasting. Only modest effects of the inhibition of IL-6 trans-signaling were observed in insulin response.

Although obesity in midlife is a risk factor for AD, weight loss is a common feature of patients, leading to poorer health outcomes and reducing quality of life (Gillette-Guyonnet et al. 2000; Aziz et al. 2008; Alford et al. 2018). Nevertheless, food intake is normal or even increased in these patients (Spindler et al. 1996; Knight et al. 2012). The underlying cause(s) of the lower body weight is still unclear. Thus, more research is necessary to understand the mechanisms behind. There are numerous similarities between the two studied animal models and AD patients; hence they could provide useful information about metabolism under AD conditions. In this regard, the 3xTg-AD model could be a suitable model; it presents the previously mentioned characteristics about AD patients and besides, it is possible to expose the mice to a HFD-obesity before the pathology and check the consequences after all.

Accordingly, changes in Aβ levels after a HFD were analyzed in the 3xTg-AD model. Surprisingly, a reduction in $A\beta_{40}$ was observed in cortex from both sexes in comparison with mice fed with control diet; besides, a reduction in $A\beta_{42}$ was also observed in both brain areas from males. Previous studies do not support an inhibitory effect of HFD in 3xTg-AD females once the pathology is already present (Knight et al. 2014; Christensen and Pike 2017); the same happens in the Tg2576 model (Elhaik Goldman et al. 2018). As stated in the Chapter 2, the putative reasons for these discrepancies are unclear: type of HFD, differences in age when starting the diet, type of housing and specific manipulations done to the animals might be relevant.

CONCLUSIONS

Tg2576

1. The Tg2576 animals showed increased perinatal and premature mortality in females, reduced body weight and increased Aβ accumulation with aging and associated neuroinflammation, especially surrounding amyloid plaques. Regarding behavioral traits, our animals showed hyperactivity, increased exploration and decreased anxiety, in a sex and age-dependent manner, as well as deficits in hippocampal-dependent learning and memory.

The inhibition of the IL-6 trans-signaling:

2. Decreased the APP-induced premature mortality in females, whereas a trend was also observed in males.
3. Did not have effects on body weight.
4. Reversed APP-induced effects on exploration and anxiety before amyloid plaques deposition in female mice only.
5. Modulated activity after amyloid plaques deposition in both sexes, reversing APP-induced hyperactivity in males and increasing it independently from *App* transgene in females.
6. Affected memory before and after amyloid plaques deposition and in both acquisition and reversal phases.
7. In females, increased total APP and its proteolytic fragments amount in hippocampus, whereas reduced Aβ levels in cortex.
8. In females, reduced the amyloid plaque deposition in both cortex and hippocampus.
9. Affected the expression of genes involved in blood vessels development and matrix organization in APP positive female mice.
10. Did not affect gliosis.

3xTg-AD

1. 3xTg-AD animals showed a mild amyloid pathology, and few plaques were detected in cortex and hippocampus of 20-24 month-old females. Accordingly, much lower Aβ levels were observed in these brain areas using ELISA in comparison with the Tg2576. An increased mortality was displayed by these mice. Regarding behavioral traits, our adult animals showed normal locomotion and anxiety and increased exploration and emotionality. Learning and memory were not affected. On the other hand, 3xTg-AD mice showed a hypermetabolic state. HFD caused obesity and endocrine and metabolic changes, and decreased $A\beta_{40}$ and $A\beta_{42}$ levels.

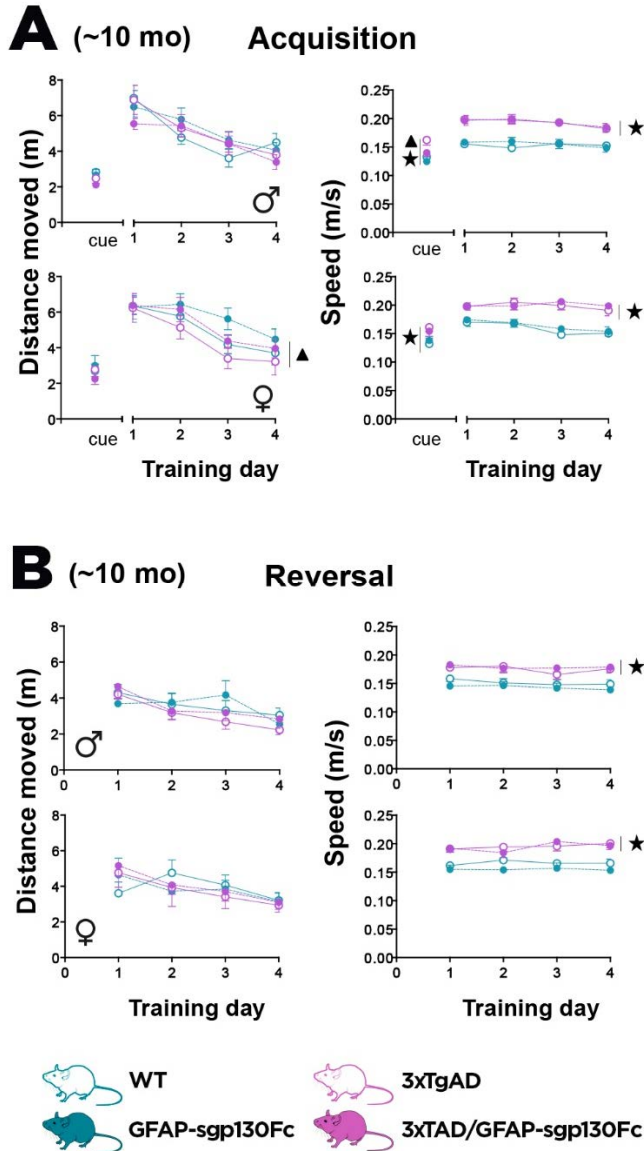The inhibition of the IL-6 trans-signaling:

2. Had sex-dependent effects on 3xTg-AD-induced premature mortality; it was detrimental in males and beneficial in females.
3. Did not have effects on body weight of animals fed with normal chow diet.
4. Reduced the 3xTg-AD-induced hyperphagia in both sexes, especially in fasting conditions.
5. Had modest effects on behavior and spatial learning and memory; at 10-11 months, decreased anxiety in males and impaired learning in females. In contrast, at 5-6 months, improved learning and short-term memory in females.
6. Potentiated the hypermetabolic state in females regardless from 3xTg-AD genotype.
7. Did not affect significantly $A\beta_{40}$ and $A\beta_{42}$ levels with either control diet or HFD.
8. Decreased the expression of the 3xTg-AD-induced hypothalamic *Il6*.
9. Mildly increased microgliosis regardless 3xTg-AD genotype in the cortex of females with HFD.

SUPPLEMENTARY DATA

Supplementary figures
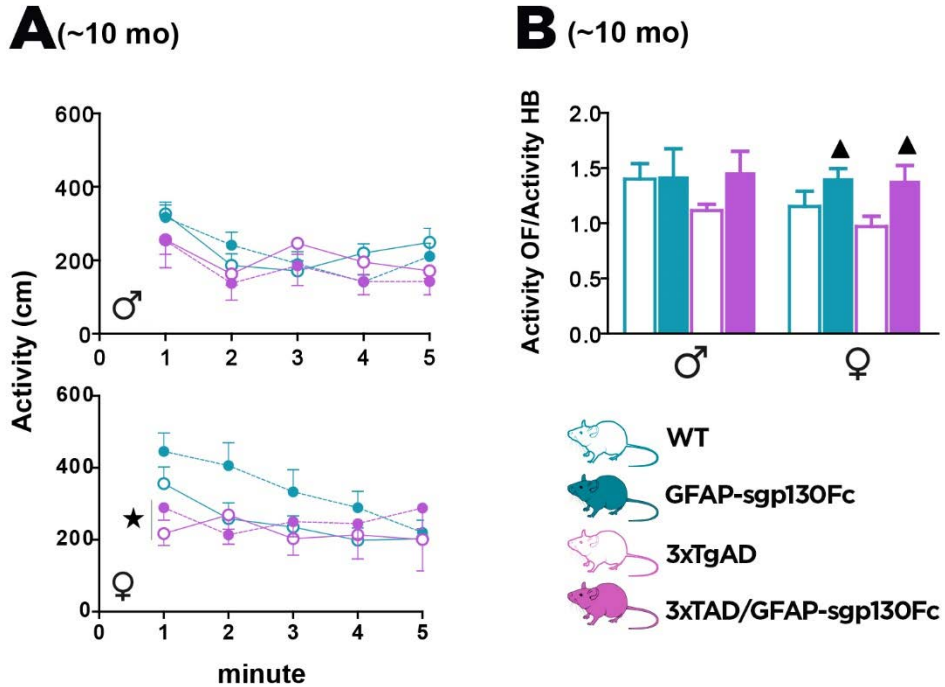
Supplementary figure 1. Horizontal activity analysis. (A) Time course of horizontal activity in OF. 3xTg-AD positive females showed lower activity during the first minute than 3xTg-AD negative, although no significant the same pattern is observed in males. The blocking of IL-6 trans-signaling trend (p = 0.053) to revert this effect in females. (B) Ratio between activity in the OF and in the HB. In females, the presence of sgp130Fc produced a higher diminution in the HB activity in females. Results are MEAN ± SEM. ★ *p≤ 0.05 vs. 3xTg-AD negative;* ▲ *p≤ 0.05 vs. sgp130Fc negative.*

Supplementary figure 2. Distance moved and swimming speed in the MWM at ~10-11 months of age. (A) During the acquisition, the inhibition of the IL-6 trans-signaling increased distance moved in females. 3xTg-AD positive animals had higher swimming speed than 3xTg-AD negative in the cue learning and during the test; the presence of sgp130Fc decreased speed in males during the cue learning. (B) During the reversal, no differences in distance were observed between genotypes. 3xTg-AD positive animals also showed increased swimming speed. Results are MEAN ± SEM. ★ *p≤ 0.05 vs. 3xTg-AD negative;* ▲ *p≤ 0.05 vs. sgp130Fc negative.*

Supplementary materials and methods

Genotyping of animals

DNA extraction and Polymerase Chain Reactions (PCRs)

A small section (~ 1mm) of tail was boiled in 100 μl of 50mM NaOH for 7 minutes and centrifuged. Then, PCR was realized immediately or the DNA extraction was stored at -20 ºC. For genotyping all the animals, three different PCRs were set up. PCR reagents as Taq Polymerase or buffer were obtained from Biotools or Thermofisher, primers and oligonucleotides are from Sigma.

Tg2576
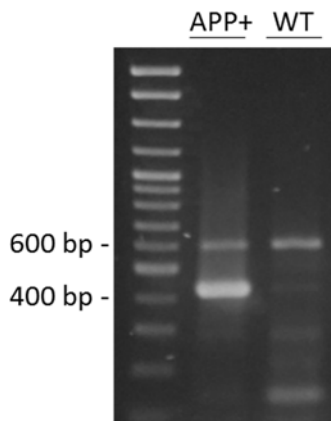
Supplementary figure 3. For APP transgene in Tg2576 animals, a single band at 600 bp should appear in WT animals. In APP positive animals an extra band at 400 bp should appear.
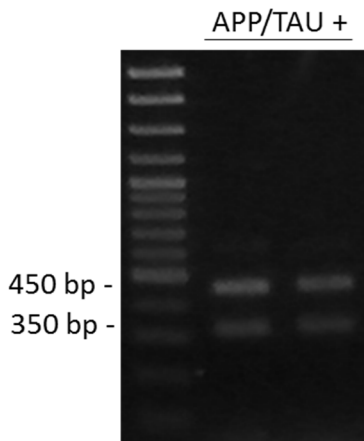
Supplementary Table 1. Primer sequences for genotyping Tg2576 animals.

Primer ID	Sequence 5' – 3'
1502	GTGGATAACCCCTCCCCCAGCCTAGACCA
1503	CTGACCACTCGACCAGGTTCTGGGT
1505	TGGTGGTAGTTGGGGTCAGC

Supplementary Table 2. PCR conditions.

Step	Temperature (Cº)	Time (s)	Cycles (n)
Initial denaturation	95	120	1
Denaturation	95	10	30
Annealing	54	10	30
Elongation	72	40	30
Final elongation	72	300	1

3xTg-AD

APP/TAU +

450 bp -
350 bp -

Supplementary figure 4. The two transgenes were detected in the same PCR procedure. APP band appears at 450 bp, whereas Tau band appears at 350 bp.
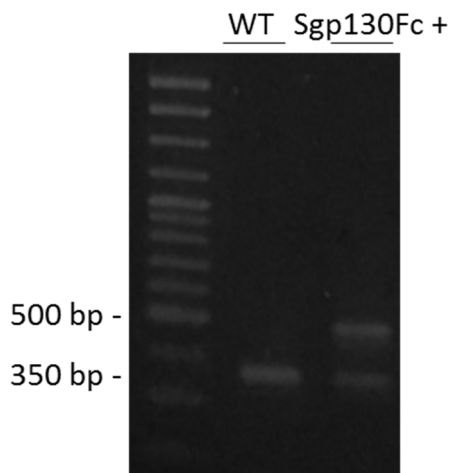
Supplementary Table 3. Primer sequences for genotyping 3xTg-AD animals.

Primer ID	Sequence 5' – 3'
App-F	GCTTGCACCAGTTCTGGATGG
App-R	GAGGTATTCAGTCATGTGCT
Tau-F	GAGGTATTCAGTCATGTGCT
Tau-R	TTCAAAGTTCACCTGATAGT

Supplementary Table 4. PCR conditions.

Step	Temperature (Cº)	Time (s)	Cycles (n)
Initial denaturation	94	300	1
Denaturation	94	30	25
Annealing	52	30	25
Elongation	72	60	25
Final elongation	72	180	1

Sgp130Fc

Supplementary figure 5. A single band appears at 500 bp, a housekeeper band were included at 350 bp.

Supplementary Table 5. Primer sequences for genotyping sgp130Fc animals.

Primer ID	Sequence 5' – 3'
Sgp130Fc-F	GAGGTGACATGTGTGGTGGTGGATGTGTC
Sgp130Fc-R	GAGAACACGTTGCCCTGCTGCCATCTAG

Supplementary Table 6. PCR conditions for the sgp130Fc PCR.

Step	Temperature (Cº)	Time (s)	Cycles (n)
Initial denaturation	95	180	1
Denaturation	95	30	34
Annealing	65	30	34
Elongation	72	60	34
Final elongation	72	600	1

REFERENCES

Adebakin A, Bradley J, Gümüsgöz S, et al (2012) Impaired satiation and increased feeding behaviour in the triple-transgenic Alzheimer's disease mouse model. PLoS One 7:. doi: 10.1371/journal.pone.0045179

Ahmed Z, Shaw G, Sharma VP, et al (2007) Actin-binding proteins coronin-1a and IBA-1 are effective microglial markers for immunohistochemistry. J Histochem Cytochem 55:687–700. doi: 10.1369/jhc.6A7156.2007

Alford S, Patel D, Perakakis N, Mantzoros CS (2018) Obesity as a risk factor for Alzheimer's disease: weighing the evidence. Obes Rev 19:269–280. doi: 10.1111/obr.12629

Allué JA, Sarasa L, Izco M, et al (2016) Outstanding phenotypic differences in the profile of amyloid-β between Tg2576 and APPswe/PS1dE9 transgenic mouse models of Alzheimer's Disease. J Alzheimer's Dis 53:773–785. doi: 10.3233/JAD-160280

Aniszewska A, Szymanski J, Winnicka MM, Turlejski K (2014) Interleukin 6 deficiency affects spontaneous activity of mice in age- and sex-dependent manner. Acta Neurobiol Exp (Wars) 74:424–432

Archer J (1973) Tests for emotionality in rats and mice: A review. Anim Behav 21:205–235

Arimon M, Takeda S, Post KL, et al (2015) Oxidative stress and lipid peroxidation are upstream of amyloid pathology. Neurobiol Dis 84:109–119. doi: 10.1016/j.nbd.2015.06.013

Armario A, Hernández J, Bluethmann H, Hidalgo J (1998) IL-6 deficiency leads to increased emotionality in mice: Evidence in transgenic mice carrying a null mutation for IL-6. J Neuroimmunol 92:160–169. doi: 10.1016/S0165-5728(98)00199-4

Arnold P, Boll I, Rothaug M, et al (2017a) Meprin metalloproteases generate biologically active soluble interleukin-6 receptor to induce trans-signaling. Sci Rep 7:44053. doi: 10.1038/srep44053

Arnold P, Otte A, Becker-Pauly C (2017b) Meprin metalloproteases: Molecular

regulation and function in inflammation and fibrosis. Biochim Biophys Acta - Mol Cell Res 1864:2096–2104. doi: 10.1016/j.bbamcr.2017.05.011

Arnone D, McIntosh AM, Ebmeier KP, et al (2012) Magnetic resonance imaging studies in unipolar depression: Systematic review and meta-regression analyses. Eur Neuropsychopharmacol 22:1–16. doi: 10.1016/j.euroneuro.2011.05.003

Aziz NA, Van Der Marck MA, Pijl H, et al (2008) Weight loss in neurodegenerative disorders. J Neurol 255:1872–1880. doi: 10.1007/s00415-009-0062-8

Baeta-Corral R, Johansson B, Giménez-Llort L (2018) Long-term treatment with low-dose caffeine worsens BPSD-Like profile in 3xTg-AD mice model of Alzheimer's disease and affects mice with normal aging. Front Pharmacol 9:79. doi: 10.3389/fphar.2018.00079

Baier PC, May U, Scheller J, et al (2009) Impaired hippocampus-dependent and -independent learning in IL-6 deficient mice. Behav Brain Res 200:192–196. doi: 10.1016/j.bbr.2009.01.013

Banks WA, Kastin AJ, Gutierrez EG (1994) Penetration of interleukin-6 across the murine blood-brain barrier. Neurosci Lett 179:53–56

Barage SH, Sonawane KD (2015) Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. Neuropeptides 52:1–18. doi: 10.1016/j.npep.2015.06.008

Baratchi S, Evans J, Tate WP, et al (2012) Secreted amyloid precursor proteins promote proliferation and glial differentiation of adult hippocampal neural progenitor cells. Hippocampus 22:1517–1527. doi: 10.1002/hipo.20988

Barron AM, Rosario ER, Elteriefi R, Pike CJ (2013) Sex-specific effects of high fat diet on indices of metabolic syndrome in 3xTg-AD mice: implications for Alzheimer's disease. PLoS One 8:. doi: 10.1371/journal.pone.0078554

Bauer J, Strauss S, Schreiter-Gasser U, et al (1991) Interleukin-6 and alpha-2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. FEBS Lett 285:111–114. doi: 10.1016/0014-5793(91)80737-N

Bauer S, Kerr BJ, Patterson PH (2007) The neuropoietic cytokine family in development, plasticity, disease and injury. Nat Rev Neurosci 8:221–232. doi: 10.1038/nrn2054

Bayer TA, Schafer S, Simons A, et al (2003) Dietary Cu stabilizes brain superoxide

dismutase 1 activity and reduces amyloid A production in APP23 transgenic mice. Proc Natl Acad Sci 100:14187–14192. doi: 10.1073/pnas.2332818100

Bazan JF (1990) Structural design and molecular evolution of a cytokine receptor superfamily. Proc Natl Acad Sci 87:6934–6938. doi: 10.1073/pnas.87.18.6934

Belfiore R, Rodin A, Ferreira E, et al (2018) Temporal and regional progression of Alzheimer's disease-like pathology in 3xTg-AD mice. Aging Cell 18:e12873. doi: 10.1111/acel.12873

Belgardt BF, Brüning JC (2010) CNS leptin and insulin action in the control of energy homeostasis. Ann N Y Acad Sci 1212:97–113. doi: 10.1111/j.1749-6632.2010.05799.x

Bell RD, Zlokovic B V. (2009) Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol 118:103–113. doi: 10.1007/s00401-009-0522-3

Benrick A, Schéle E, Pinnock SB, et al (2009) Interleukin-6 gene knockout influences energy balance regulating peptides in the hypothalamic paraventricular and supraoptic nuclei. J Neuroendocrinol 21:620–628. doi: 10.1111/j.1365-2826.2009.01879.x

Benzing WC, Wujek JR, Ward EK, et al (1999) Evidence for glial-mediated inflammation in aged APPSW transgenic mice. Neurobiol Aging 20:581–589. doi: 10.1016/S0197-4580(99)00065-2

Bermejo P, Martín-Aragón S, Benedí J, et al (2008) Differences of peripheral inflammatory markers between mild cognitive impairment and Alzheimer's disease. Immunol Lett 117:198–202. doi: 10.1016/j.imlet.2008.02.002

Bertram L, Lill CM, Tanzi RE (2010) The genetics of Alzheimer disease: Back to the future. Neuron 68:270–281. doi: 10.1016/j.neuron.2010.10.013

Bertram L, Tanzi RE (2004) The current status of Alzheimer ' s disease genetics : what do we tell the patients ? 50:385–396. doi: 10.1016/j.phrs.2003.11.018

Bethin KE, Vogt SK, Muglia LJ (2000) Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. Proc Natl Acad Sci 97:9317–9322. doi: 10.1073/pnas.97.16.9317

Bien J, Jefferson T, Čaušević M, et al (2012) The metalloprotease meprin β generates

amino terminal-truncated amyloid β peptide species. J Biol Chem 287:33304–33313. doi: 10.1074/jbc.M112.395608

Billings LM, Oddo S, Green KN, et al (2005) Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 45:675–688. doi: 10.1016/j.neuron.2005.01.040

Bizon J, Prescott S, Nicolle MM (2007) Intact spatial learning in adult Tg2576 mice. Neurobiol Aging 28:440–446. doi: 10.1016/j.neurobiolaging.2006.01.004

Blacker D, Haines JL, Rodes L, et al (1997) ApoE-4 and age at onset of Alzheimer's disease: The NIMH genetics initiative. Neurology 48:139–147. doi: 10.1212/WNL.48.1.139

Bolin LM, Verity AN, Silver JE, et al (1995) Interleukin-6 production by Schwann Cells and induction in sciatic nerve injury. J Neurochem 64:850–858. doi: 10.1046/j.1471-4159.1995.64020850.x

Bolin LM, Zhaung A, Strychkarska-Orczyk I, et al (2005) Differential inflammatory activation of IL-6 (-/-) astrocytes. Cytokine 30:47–55. doi: 10.1016/j.cyto.2004.11.007

Bonni A, Sun Y, Nadal-vicens M, et al (1997) Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway. Science (80-) 278:477–483. doi: 10.1126/science.278.5337.477

Braak H, Braak E (1997) Frequency of stages of Alzheimer-related lesions in different age categories: concurrences and cautions. Neurobiol Aging 18:351–357. doi: 10.1016/s0197-4580(97)00052-3

Braida D, Sacerdote P, Panerai AE, et al (2004) Cognitive function in young and adult IL (interleukin)-6 deficient mice. Behav Brain Res 153:423–429. doi: 10.1016/j.bbr.2003.12.018

Braun O, Dewitz C, Möller-Hackbarth K, et al (2013) Effects of blockade of peripheral Interleukin-6 trans-signaling on hippocampus-dependent and independent memory in mice. J Interf Cytokine Res 33:254–260. doi: 10.1089/jir.2012.0096

Brenner M, Messing A (1996) GFAP transgenic mice. Methods A Companion to Methods Enzymol 10:351–364. doi: 10.1006/meth.1996.0113

Brown GR, Nemes C (2008) The exploratory behaviour of rats in the hole-board

apparatus: Is head-dipping a valid measure of neophilia? Behav Processes 78:442–448. doi: 10.1016/j.beproc.2008.02.019

Brugg B, Dubreuil YL, Huber G, et al (1995) Inflammatory processes induce beta-amyloid precursor protein changes in mouse brain. Proc Natl Acad Sci 92:3032–3035. doi: 10.1073/pnas.92.7.3032

Brunello AG, Weissenberger J, Kappeler A, et al (2000) Astrocytic alterations in interleukin-6/soluble interleukin-6 receptor α double-transgenic mice. Am J Pathol 157:1485–1493. doi: 10.1016/S0002-9440(10)64787-6

Burns A, Iliffe S (2009) Alzheimer's disease. Br Med J 338:467471. doi: 10.1016/j.med.2019.03.012

Burton MD, Rytych JL, Freund GG, Johnson RW (2013) Central inhibition of interleukin-6 trans-signaling during peripheral infection reduced neuroinflammation and sickness in aged mice. Brain Behav Immun 30:66–72. doi: 10.1016/j.bbi.2013.01.002

Burton MD, Sparkman NL, Johnson RW (2011) Inhibition of interleukin-6 trans-signaling in the brain facilitates recovery from lipopolysaccharide-induced sickness behavior. J Neuroinflammation 8:1–13. doi: 10.1186/1742-2094-8-54

Bush AI, Tanzi RE (2008) Therapeutics for Alzheimer's disease based on the Metal Hypothesis. Neurotherapeutics 5:421–432. doi: 10.1016/j.nurt.2008.05.001

Butterweck V, Prinz S, Schwaninger M (2003) The role of interleukin-6 in stress-induced hyperthermia and emotional behaviour in mice. Behav Brain Res 144:49–56. doi: 10.1016/S0166-4328(03)00059-7

Caccamo A, Branca C, Talboom JS, et al (2015) Reducing ribosomal protein S6 Kinase 1 expression improves spatial memory and synaptic plasticity in a mouse model of Alzheimer's Disease. J Neurosci 35:14042–14056. doi: 10.1523/jneurosci.2781-15.2015

Cai D, Yuan M, Frantz DF, et al (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-β and NF-κB. Nat Med 11:183–190. doi: 10.1038/nm1166

Cai Z, Hussain MD, Yan LJ (2014) Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer's disease. Int J Neurosci 124:307–321. doi: 10.3109/00207454.2013.833510

Callahan MJ, Lipinski WJ, Bian F, et al (2001) Augmented senile plaque load in aged female β-amyloid precursor protein-transgenic mice. Am J Pathol 158:1173–1177. doi: 10.1016/S0002-9440(10)64064-3

Calsolaro V, Edison P (2016) Neuroinflammation in Alzheimer's disease: Current evidence and future directions. Alzheimer's Dement 12:719–732. doi: 10.1016/j.jalz.2016.02.010

Campbell IL, Abraham CR, Masliah E, et al (1993) Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. Proc Natl Acad Sci 90:10061–10065. doi: 10.1073/pnas.90.21.10061

Campbell IL, Erta M, Lim SL, et al (2014) Trans-signaling is a dominant mechanism for the pathogenic actions of interleukin-6 in the brain. J Neurosci 34:2503–2513. doi: 10.1523/JNEUROSCI.2830-13.2014

Cañete T, Blázquez G, Tobeña A, et al (2015) Cognitive and emotional alterations in young Alzheimer's disease (3xTgAD) mice: Effects of neonatal handling stimulation and sexual dimorphism. Behav Brain Res 281:156–171. doi: 10.1016/j.bbr.2014.11.004

Carlson GA, Borchelt DR, Dake A, et al (1997) Genetic modification of the phenotypes produced by amyloid precursor protein overexpression in transgenic mice. Hum Mol Genet 6:1951–1959. doi: 10.1093/HMG/6.11.1951

Carson MJ, Doose JM, Melchior B, et al (2003) CNS immune privilege: hiding in plain sight. Immunol Rev 213:48–65. doi: 10.1111/j.1600-065X.2006.00441.x.CNS

Castell J V, Geiger T, Gross V, et al (1988) Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat. Eur J Biochem 177:357–361. doi: 10.1111/j.1432-1033.1988.tb14384.x

Castillo GM, Ngo C, Cummings J, et al (1997) Perlecan binds to the β-amyloid proteins (Aβ) of Alzheimer's Disease, accelerates Aβ fibril formation, and maintains Aβ fibril stability. J Neurochem 69:2452–2465. doi: 10.1046/j.1471-4159.1997.69062452.x

Chakrabarty P, Jansen-West K, Beccard A, et al (2010) Massive gliosis induced by interleukin-6 suppresses Aβ deposition in vivo: evidence against inflammation as a driving force for amyloid deposition. FASEB J 24:548–559. doi: 10.1096/fj.09-141754

Chan J, Jones NC, Bush AI, et al (2015) A mouse model of Alzheimer's disease displays increased susceptibility to kindling and seizure-associated death. Epilepsia 56:e73–e77. doi: 10.1111/epi.12993

Chapman PF, Hsiao K, White GL, et al (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nat Neurosci 2:271–276. doi: 10.1038/6374

Chikuma T, Yoshimoto T, Ohba M, et al (2009) Interleukin-6 induces prostaglandin E2 synthesis in mouse astrocytes. J Mol Neurosci 39:175–184. doi: 10.1007/s12031-009-9187-6

Chong Y (1997) Effect of a carboxy-terminal fragment of the Alzheimer's Amyloid Precursor Protein on expression of proinflammatory cytokines in rat glial cells. Life Sci 61:2323–2333

Christensen A, Pike CJ (2017) Age-dependent regulation of obesity and Alzheimer-related outcomes by hormone therapy in female 3xTg-AD mice. PLoS One 12:1–23. doi: 10.1371/journal.pone.0178490

Chung CD, Liao J, Liu B, et al (1997) Specific inhibition of Stat3 signal transduction by PIAS3. Science (80-) 278:1803–1805

Clarris HJ, Cappai R, Heffernan D, et al (2002) Identification of heparin-binding domains in the amyloid precursor protein of Alzheimer's disease by deletion mutagenesis and peptide mapping. J Neurochem 68:1164–1172. doi: 10.1016/S0025-7125(16)31188-9

Clarris HJ, Key B, Beyreuther K, et al (1995) Expression of the amyloid protein precursor of Alzheimer's disease in the developing rat olfactory system. Dev Brain Res 88:87–95. doi: 10.1016/0165-3806(95)00083-P

Collins SR, Douglass A, Vale RD, Weissman JS (2004) Mechanism of prion propagation: Amyloid growth occurs by monomer addition. PLoS Biol 2:. doi: 10.1371/journal.pbio.0020321

Combarros O, Infante J, Llorca J, et al (2005) Interaction between interleukin-6 and intercellular adhesion molecule-1 genes and Alzheimer's disease risk. J Neurol 252:485–487. doi: 10.1007/s00415-005-0658-6

Comes G (2017) Influece of Metallothionein-1 in the Tg2576 mouse model of Alzhimer's disease. Universitat Autònoma de Barcelona

Comes G, Manso Y, Escrig A, et al (2017) Influence of transgenic Metallothionein-1 on gliosis, CA1 neuronal loss, and brain metal levels of the Tg2576 mouse model of Alzheimer's disease. Int J Mol Sci 18:251. doi: 10.3390/ijms18020251

Congdon EE, Sigurdsson EM (2018) Tau-targeting therapies for Alzheimer disease. Nat Rev Neurol 14:399–415. doi: 10.1038/s41582-018-0013-z

Cuevas-Olguin R, Esquivel-Rendon E, Vargas-Mireles J, et al (2017) Interleukin 6 trans-signaling regulates basal synaptic transmission and sensitivity to pentylenetetrazole-induced seizures in mice. Synapse 71:1–11. doi: 10.1002/syn.21984

Cui H, Freeman C, Jacobson GA, Small DH (2013) Proteoglycans in the central nervous system: Role in development, neural repair, and Alzheimer's disease. IUBMB Life 65:108–120. doi: 10.1002/iub.1118

Dalrymple SA, Lucian LA, Slattery R, et al (1995) Interleukin-6-deficient mice are highly susceptible to Listeria monocytogenes infection: Correlation with inefficient neutrophilia. Infect Immun 63:2262–2268. doi: 10.1007/s12371-011-0040-2

Dantzer R (2009) Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am 29:247–264. doi: 10.2217/FON.09.6.Dendritic

Dawkins E, Small DH (2014) Insights into the physiological function of the β-amyloid precursor protein: Beyond Alzheimer's disease. J Neurochem 129:756–769. doi: 10.1111/jnc.12675

De Benedetti F, Rucci N, Del Fattore A, et al (2006) Impaired skeletal development in interleukin-6-transgenic mice: A model for the impact of chronic inflammation on the growing skeletal system. Arthritis Rheum 54:3551–3563. doi: 10.1002/art.22175

De Luca G, Di Giorgio RM, Macaione S, et al (2004) Susceptibility to audiogenic seizure and neurotransmitter amino acid levels in different brain areas of IL-6-deficient mice. Pharmacol Biochem Behav 78:75–81. doi: 10.1016/j.pbb.2004.02.004

De Sarro G, Russo E, Ferreri G, et al (2004) Seizure susceptibility to various convulsant stimuli of knockout interleukin-6 mice. Pharmacol Biochem Behav 77:761–766. doi: 10.1016/j.pbb.2004.01.012

De Silva HAR, Jen A, Wickenden C, et al (1997) Cell-specific expression of β-

amyloid precursor protein isoform mRNAs and proteins in neurons and astrocytes. Mol Brain Res 47:147–156. doi: 10.1016/S0169-328X(97)00045-4

De Strooper B (2010) Proteases and proteolysis in Alzheimer Disease: a multifactorial view on the disease process. Physiol Rev 90:465–494. doi: 10.1152/physrev.00023.2009

Deacon RMJ, Cholerton LL, Talbot K, et al (2008) Age-dependent and -independent behavioral deficits in Tg2576 mice. Behav Brain Res 189:126–138. doi: 10.1016/j.bbr.2007.12.024

Del Bo R, Angeretti N, Lucca E, et al (1995) Reciprocal control of inflammatory cytokines, IL-1 and IL-6, and β-amyloid production in cultures. Neurosci Lett 188:70–74

Demars MP, Bartholomew A, Strakova Z, Lazarov O (2011) Soluble amyloid precursor protein: A novel proliferation factor of adult progenitor cells of ectodermal and mesodermal origin. Stem Cell Res Ther 2:36. doi: 10.1186/scrt77

Denenberg VH (1969) Open-Field behavior in the rat. what does it mean? Ann N Y Acad Sci 159:852–859

Di Gregorio GB, Hensley L, Lu T, et al (2004) Lipid and carbohydrate metabolism in mice with a targeted mutation in the IL-6 gene: absence of development of age-related obesity. Am J Physiol Metab 287:E182–E187. doi: 10.1152/ajpendo.00189.2003

Di Santo E, Alonzi T, Fattori E, et al (1996) Overexpression of interleukin-6 in the central nervous system of transgenic mice increases central but not systemic proinflammatory cytokine production. Brain Res 740:239–244. doi: 10.1016/S0006-8993(96)00865-7

Diamant M, Rieneck K, Mechti N, et al (1997) Cloning and expression of an alternatively spliced mRNA encoding a soluble form of the human interleukin-6 signal transducer gp130. FEBS Lett 412:379–384. doi: 10.1016/S0014-5793(97)00750-3

Dickson TC, Vickers JC (2001) The morphological phenotype of β-amyloid plaques and associated neuritic changes in Alzheimer's disease. Neuroscience 105:99–107. doi: 10.1016/S0306-4522(01)00169-5

DiSabato D, Quan N, Godbout JP (2016) Neuroinflammation: the devil is in the

details. J Neurochem 139:136–153. doi: 10.1002/jhm.463

Dittrich E, Rose-John S, Gerhartz C, et al (1994) Identification of a region within the cytoplasmic domain of the Interleukin-6 signal transducer gp130 important for ligand-induced endocytosis of the IL-6 receptor. J Biol Chem 269:19014–19020. doi: 10.1111/j.1749-6632.1995.tb32350.x

Dournaud P, Delaere P, Hauw JJ, Epelbaum J (1995) Differential correlation between neurochemical deficits, neuropathology, and cognitive status in Alzheimer's disease. Neurobiol Aging 16:817–823. doi: 10.1016/0197-4580(95)00086-T

Drummond E, Wisniewski T (2017) Alzheimer's disease: experimental models and reality. Acta Neuropathol 133:155–175. doi: 10.1007/s00401-016-1662-x

Ebert U, Kirch W (1998) Scopolamine model of dementia: Electroencephalogram findings and cognitive performance. Eur J Clin Invest 28:944–949. doi: 10.1046/j.1365-2362.1998.00393.x

Eden E, Navon R, Steinfeld I, et al (2009) GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics 10:1–7. doi: 10.1186/1471-2105-10-48

El Khoury J, Toft M, Hickman SE, et al (2007) Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. Nat Med 13:432–438. doi: 10.1038/nm1555

Elhaik Goldman S, Goez D, Last D, et al (2018) High-fat diet protects the blood–brain barrier in an Alzheimer's disease mouse model. Aging Cell 17:. doi: 10.1111/acel.12818

Ellingsgaard H, Ehses JA, Hammar EB, et al (2008) Interleukin-6 regulates pancreatic alfa-cell mass expansion. Proc Natl Acad Sci 105:13163–13168. doi: 10.1073/pnas.0801059105

Erta M, Giralt M, Esposito FL, et al (2015) Astrocytic IL-6 mediates locomotor activity, exploration, anxiety, learning and social behavior. Horm Behav 73:64–74. doi: 10.1016/j.yhbeh.2015.06.016

Erta M, Quintana A, Hidalgo J (2012) Interleukin-6, a major cytokine in the central nervous system. Int J Biol Sci 8:1254–1266. doi: 10.7150/ijbs.4679

Eulenfeld R, Dittrich A, Khouri C, et al (2012) Interleukin-6 signalling: More than

Jaks and STATs. Eur J Cell Biol 91:486–495. doi: 10.1016/j.ejcb.2011.09.010

Eulenfeld R, Schaper F (2009) A new mechanism for the regulation of Gab1 recruitment to the plasma membrane. J Cell Sci 122:55–64. doi: 10.1242/jcs.037226

Fanslow WC, Clifford KN, Park LS, et al (1991) Regulation of alloreactivity in vivo by IL-4 and the soluble IL- 4 receptor. J Immunol 147:535–540

Fanslow WC, Sims JE, Sassenfeld H, et al (1990) Regulation of alloreactivity in vivo by a soluble form of the interleukin-1 receptor. Science (80-) 248:739–742. doi: 10.1126/science.2139736

Femminella GD, Thayanandan T, Calsolaro V, et al (2018) Imaging and molecular mechanisms of Alzheimer's disease: A review. Int J Mol Sci 19:. doi: 10.3390/ijms19123702

Fernández-Gayol O, Sanchis P, Aguilar K, et al (2019) Different responses to a high-fat diet in IL-6 conditional knock-out mice driven by constitutive GFAP-Cre and Synapsin 1-Cre expression. Neuroendocrinology. doi: 10.1159/000496845

Ferrer B, Navia B, Giralt M, et al (2014) Muscle-specific interleukin-6 deletion influences body weight and body fat in a sex-dependent manner. Brain Behav Immun 40:121–130. doi: 10.1016/j.bbi.2014.03.001

Fisher FM, Chui PC, Antonellis PJ, et al (2010) Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes 59:2781–2789. doi: 10.2337/db10-0193

Fisher FM, Maratos-Flier E (2016) Understanding the physiology of FGF21. Annu Rev Physiol 78:223–241. doi: 10.1146/annurev-physiol-021115-105339

Forloni G, Balducci C (2018) Alzheimer's disease, oligomers, and inflammation. J Alzheimer's Dis 62:1261–1276. doi: 10.3233/JAD-170819

Frautschy SA, Yang F, Irrizarry M, et al (1998) Microglial response to amyloid plaques in APPsw transgenic mice. Am J Pathol 152:307–317

Freude S, Hettich MM, Schumann C, et al (2009) Neuronal IGF-1 resistance reduces Aβ accumulation and protects against premature death in a model of Alzheimer's disease. FASEB J 23:3315–3324. doi: 10.1096/fj.09-132043

Frigerio CS, Piscopo P, Calabrese E, et al (2005) PEN-2 gene mutation in a familial Alzheimer's disease case. J Neurol 252:1033–1036. doi: 10.1007/s00415-005-0799-7

Fuhrmann M, Bittner T, Jung CKE, et al (2010) Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. Nat Neurosci 13:411–413. doi: 10.1038/nn.2511

Fukada T, Hibi M, Yamanaka Y, et al (1996) Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: Involvement of STAT3 in anti-apoptosis. Immunity 5:449–460. doi: 10.1016/S1074-7613(00)80501-4

Garbers C, Heink S, Korn T, Rose-John S (2018) Interleukin-6: designing specific therapeutics for a complex cytokine. Nat Rev Drug Discov 17:395–412. doi: 10.1038/nrd.2018.45

Garbers C, Jänner N, Chalaris A, et al (2011) Species specificity of ADAM10 and ADAM17 proteins in interleukin-6 (IL-6) trans-signaling and novel role of ADAM10 in inducible IL-6 receptor shedding. J Biol Chem 286:14804–14811. doi: 10.1074/jbc.M111.229393

Gerhartz C, Heesel B, Sasse J, et al (1996) Differential activation of acute phase response factor/Stat3 and Stat1 via the cytoplasmic domain of the Interleukin 6 signal transducer gp130. J Biol Chem 271:12991–12998. doi: 10.1074/jbc.271.22.12999

Gillette-Guyonnet S, Nourhashemi F, Andrieu S, et al (2000) Weight loss in Alzheimer disease. Am J Clin Nutr 71:637S-642S. doi: 10.1093/ajcn/71.2.637s

Giménez-Llort L, Arranz L, Maté I, De La Fuente M (2008) Gender-specific neuroimmunoendocrine aging in a triple-transgenic 3×Tg-AD mouse model for Alzheimer's disease and its relation with longevity. Neuroimmunomodulation 15:331–343. doi: 10.1159/000156475

Giménez-Llort L, Blázquez G, Cañete T, et al (2007) Modeling behavioral and neuronal symptoms of Alzheimer's disease in mice: A role for intraneuronal amyloid. Neurosci Biobehav Rev 31:125–147. doi: 10.1016/j.neubiorev.2006.07.007

Glenner GG, Wong CW (1984) Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120:885–890. doi: 10.1016/j.bbrc.2012.08.020

Goate A, Chartier-Harlin M-C, Mullan M, et al (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349:704–706

Gopinathan G, Milagre C, Pearce OMT, et al (2015) Interleukin-6 stimulates defective angiogenesis. Cancer Res 75:3098–3107. doi: 10.1158/0008-5472.CAN-15-1227

Grötzinger J, Kernebeck T, Kallen KJ, Rose-John S (1999) IL-6 type cytokine receptor complexes: Hexamer, tetramer or both? Biol Chem 380:803–813. doi: 10.1515/BC.1999.100

Grötzinger J, Kurapkat G, Wollmer A, et al (1997) The family of the IL-6-Type Cytokines : specificity and promiscuity of the receptor complexes. Proteins 27:96–109

Guerreiro R, Wojtas A, Bras J, et al (2013) TREM2 variants in Alzheimer's Disease. N Engl J Med 368:117–127. doi: 10.1056/NEJMoa1211851

Guglielmotto M, Giliberto L, Tamagno E, Tabaton M (2010) Oxidative stress mediates the pathogenic effect of different Alzheimer ' s disease risk factors. Front Aging Neurosci 2:1–8. doi: 10.3389/neuro.24.003.2010

Guillemin G, Boussin FD, Le Grand R, et al (1996) Granulocyte macrophage colony stimulating factor stimulates in vitro proliferation of astrocytes derived from simian mature brains. Glia 16:71–80. doi: 10.1002/(SICI)1098-1136(199601)16:1<71::AID-GLIA8>3.0.CO;2-E

Guix FX, Wahle T, Vennekens K, et al (2012) Modification of γ-secretase by nitrosative stress links neuronal ageing to sporadic Alzheimer's disease. EMBO Mol Med 4:660–673. doi: 10.1002/emmm.201200243

Guo T, Noble W, Hanger DP (2017) Roles of tau protein in health and disease. Acta Neuropathol 133:665–704. doi: 10.1007/s00401-017-1707-9

Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol. doi: 10.1038/nrm2101

Haddick PCG, Larson JL, Rathore N, et al (2017) A common variant of IL-6R is associated with elevated IL-6 pathway activity in Alzheimer's disease brains. J Alzheimer's Dis 56:1037–1054. doi: 10.3233/JAD-160524

Hardy JA, Higgins GA (1992) Alzheimer ' s Disease : The Amyloid Cascade Hypothesis. Science (80-) 256:184–185

Harkany T, O'Mahony S, Kelly JP, et al (1998) beta-Amyloid(Phe(SO 3 H) 24)25–35

in rat nucleus basalis induces behavioral dysfunctions, impairs learning and memory and disrupts cortical cholinergic innervation. Behav Brain Res 90:133–145

He F, Ge W, Martinowich K, et al (2005) A positive autoregulatory loop of Jak-STAT signaling controls the onset of astrogliogenesis. Nat Neurosci 8:616–625. doi: 10.1038/nn1440

Heber S, Herms J, Gajic V, et al (2000) Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. J Neurosci 20:7951–7963

Hee B, Zhou M, Johnson AW, et al (2015) Contribution of reactive oxygen species to cerebral amyloid angiopathy, vasomotor dysfunction, and microhemorrhage in aged Tg2576 mice. Proc Natl Acad Sci USA 112:881–890. doi: 10.1073/pnas.1414930112

Heink S, Yogev N, Garbers C, et al (2016) Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic TH17 cells. Nat Immunol 18:74–85. doi: 10.1038/ni.3632

Heinrich PC, Behrmann I, Haan S, et al (2003) Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374:1–20. doi: 10.1042/BJ20030407

Heinrich PC, Castell J V, Andus T (1990) Interleukin-6 and the acute phase response. Biochem J 265:621–636

Heneka MT, Carson MJ, Khoury J El, et al (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14:388–405. doi: 10.1016/S1474-4422(15)70016-5

Heppner FL, Ransohoff RM, Becher B (2015) Immune attack: the role of inflammation in Alzheimer disease. Nat Rev Neurosci 16:358–372. doi: 10.1038/nrn3880

Herrmann JE, Imura T, Song B, et al (2008) STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. J Neurosci 28:7231–7243. doi: 10.1523/jneurosci.1709-08.2008

Herrup K (2015) The case for rejecting the amyloid cascade hypothesis. Nat Neurosci 18:794–799. doi: 10.1038/nn.4017

Heyser CJ, Masliah E, Samimi a, et al (1997) Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin 6 in the brain. Proc Natl Acad Sci U S A 94:1500–1505. doi: 10.1073/pnas.94.4.1500

Hidalgo J, Florit S, Giralt M, et al (2010) Transgenic mice with astrocyte-targeted production of interleukin-6 are resistant to high-fat diet-induced increases in body weight and body fat. Brain Behav Immun 24:119–126. doi: 10.1016/j.bbi.2009.09.002

Hippius H, Neundörfer G (2003) The discovery of Alzheimer's disease. Dialogues Clin Neurosci 5:101–108. doi: 10.1016/S0140-6736(52)90595-3

Hirano T, Taga T, Nakano N, et al (1985) Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2). Proc Natl Acad Sci U S A 82:5490–4

Hirata-Fukae C, Li HF, Hoe HS, et al (2008) Females exhibit more extensive amyloid, but not tau, pathology in an Alzheimer transgenic model. Brain Res 1216:92–103. doi: 10.1016/j.brainres.2008.03.079

Hoe HS, Fu Z, Makarova A, et al (2009) The effects of amyloid precursor protein on postsynaptic composition and activity. J Biol Chem 284:8495–8506. doi: 10.1074/jbc.M900141200

Hsiao K, Chapman P, Nilsen S, et al (1996) Correlative memory deficits, A-beta elevation, and amyloid plaques in transgenic mice. Science (80-) 274:99–102

Hsu MP, Frausto R, Rose-John S, Campbell IL (2015) Analysis of IL-6/gp130 family receptor expression reveals that in contrast to astroglia, microglia lack the oncostatin M receptor and functional responses to oncostatin M. Glia 63:132–141. doi: 10.1002/glia.22739

Huber CM, Yee C, May T, et al (2018) Cognitive decline in preclinical Alzheimer's Disease: Amyloid-Beta versus Tauopathy. J Alzheimer's Dis 61:265–281. doi: 10.3233/JAD-170490

Hüll M, Strauss S, Berger M, et al (1996) The participation of interleukin-6 , a stress-inducible cytokine , in the pathogenesis of Alzheimer ' s disease. Behav Brain Res 78:37–41

Hunter CA, Jones SA (2015) IL-6 as a keystone cytokine in health and disease. Nat Immunol 16:448–457. doi: 10.1038/ni.3153

Hurst SM, Wilkinson TS, Mcloughlin RM, et al (2001) IL-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. Immunity 14:705–714

Iadanza MG, Jackson MP, Hewitt EW, et al (2018) A new era for understanding amyloid structures and disease. Nat Rev Mol Cell Biol 19:755–773. doi: 10.1038/s41580-018-0060-8

Ishii M, Wang G, Racchumi G, et al (2014) Transgenic mice overexpressing amyloid precursor protein exhibit early metabolic deficits and a pathologically low leptin state associated with hypothalamic dysfunction in arcuate neuropeptide Y neurons. J Neurosci 34:9096–9106. doi: 10.1523/JNEUROSCI.0872-14.2014

Ittner LM, Götz J (2011) Amyloid-β and tau--a toxic pas de deux in Alzheimer's disease. Nat Rev Neurosci 12:65–72. doi: 10.1038/nrn2967

Jana M, Palencia CA, Pahan K (2008) Fibrillar amyloid-β peptides activate microglia via TLR2: Implications for Alzheimer's Disease. J Immunol 181:7254–7262. doi: 10.4049/jimmunol.181.10.7254

Jankowsky JL, Zheng H (2017) Practical considerations for choosing a mouse model of Alzheimer's disease. Mol Neurodegener 12:1–22. doi: 10.1186/s13024-017-0231-7

Jefferson T, Čaušević M, Auf Dem Keller U, et al (2011) Metalloprotease meprin β generates nontoxic N-terminal amyloid precursor protein fragments in vivo. J Biol Chem 286:27741–27750. doi: 10.1074/jbc.M111.252718

Jones SA (2005) Directingtransition from innate to acquired immunity: defining a role for IL-6. J Immunol 175:3463–3468. doi: 10.4049/jimmunol.175.6.3463

Jostock T, Müllberg J, Özbek S, et al (2001) Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. Eur J Biochem 268:160–167. doi: 10.1046/j.1432-1327.2001.01867.x

Kametani F, Hasegawa M (2018) Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. Front Neurosci 12:. doi: 10.3389/fnins.2018.00025

Kane AE, Shin S, Wong AA, et al (2018) Sex differences in healthspan predict lifespan in the 3xTg-AD Mouse model of Alzheimer's Disease. Front Aging Neurosci 10:1–10. doi: 10.3389/fnagi.2018.00172

Kang J, Hans-Georg L, Unterbeck A, et al (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325:733–736

Karch CM, Goate AM (2015) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry 77:43–51. doi: 10.1016/j.biopsych.2014.05.006.Alzheimer

Keane TM, Goodstadt L, Danecek P, et al (2011) Mouse genomic variation and its effect on phenotypes and gene regulation. Nature 477:289–294. doi: 10.1038/nature10413

Keren-Shaul H, Spinrad A, Weiner A, et al (2017) A unique microglia type associated with restricting development of Alzheimer's disease. Cell 169:1276-1290.e17. doi: 10.1016/j.cell.2017.05.018

Kim HJ, Higashimori T, Park SY, et al (2004) Differential effects of Interleukin-6 and -10 on skeletal muscle and liverinsulin action in vivo. Diabetes 53:1060–1067. doi: 10.2337/diabetes.53.4.1060

Kim M, Suh J, Romano D, et al (2009) Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene attenuate α-secretase activity. Hum Mol Genet 18:3987–3996. doi: 10.1093/hmg/ddp323

King DL, Arendash GW (2002) Behavioral characterization of the Tg2576 transgenic model of Alzheimer's disease through 19 months. Physiol Behav 75:627–642. doi: 10.1016/S0031-9384(02)00639-X

Kishimoto T, Akira S, Narazaki M, Taga T (1995) Interleukin-6 family of cytokines and gp130. Blood 86:1243–1254

Klein MA, Möller CJ, Jones LL, et al (1997) Impaired neuroglial activation in interleukin-6 deficient mice. Glia 19:227–233. doi: doi:10.1002/(SICI)1098-1136(199703)19:3<227::AID-GLIA5>3.0.CO;2-W

Kleinridders A, Ferris HA, Cai W, Kahn CR (2014) Insulin action in brain regulates systemic metabolism and brain function. Diabetes 63:2232–2243. doi: 10.2337/db14-0568

Klohs J, Rudin M, Shimshek DR, Beckmann N (2014) Imaging of cerebrovascular pathology in animal models of Alzheimer's disease. Front Aging Neurosci 6:1–30. doi: 10.3389/fnagi.2014.00032

Knight EM, Martins IVA, Gümüsgöz S, et al (2014) High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice isindependent of changes in amyloid and tau pathology. Neurobiol Aging 35:1821–1832. doi: 10.1016/j.neurobiolaging.2014.02.010

Knight EM, Verkhratsky A, Luckman SM, et al (2012) Hypermetabolism in a triple-transgenic mouse model of Alzheimer's disease. Neurobiol Aging 33:187–193. doi: 10.1016/j.neurobiolaging.2010.02.003

Koivisto AM, Helisalmi S, Pihlajamäki J, et al (2005) Interleukin-6 promoter polymorphism and late-onset Alzheimer's disease in the Finnish population. J Neurogenet 19:155–161. doi: 10.1080/01677060600569721

Kopf M, Baumann H, Freer G, et al (1994) Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 368:339–342. doi: 10.1038/368339a0

Kordula T, Rydel RE, Brigham EF, et al (1998) Oncostatin M and the interleukin-6 and soluble interleukin-6 receptor complex regulate α1-antichymotrypsin expression in human cortical astrocytes. J Biol Chem 273:4112–4118. doi: 10.1074/jbc.273.7.4112

Kraakman MJ, Kammoun HL, Allen TL, et al (2015) Blocking IL-6 trans-signaling prevents high-fat diet-induced adipose tissue macrophage recruitment but does not improve insulin resistance. Cell Metab 21:403–416. doi: 10.1016/j.cmet.2015.02.006

Kraft AW, Hu X, Yoon H, et al (2013) Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1 mice. FASEB J 27:187–198. doi: 10.1096/fj.12-208660

Kreutzberg GW (1996) Microglia: A sensor for pathological events in the CNS. Trends Neurosci 19:312–318. doi: 10.1016/0166-2236(96)10049-7

Krezowski J, Knudson D, Ebeling C, et al (2004) Identification of loci determining susceptibility to the lethal effects of amyloid precursor protein transgene overexpression. Hum Mol Genet 13:1989–1997. doi: 10.1093/hmg/ddh210

LaFerla FM, Tinkle BT, Bieberich CJ, et al (1995) The Alzheimer's A beta peptide induces neurodegeneration and apoptotic cell death in transgenic mice. Nat Genet 9:21–30. doi: 10.1038/ng0195-21

Lalonde R, Lewis TL, Strazielle C, et al (2003) Transgenic mice expressing the

βAPP695SWE mutation: Effects on exploratory activity, anxiety, and motor coordination. Brain Res 977:38–45. doi: 10.1016/S0006-8993(03)02694-5

Lassalle JM, Halley H, Daumas S, et al (2008) Effects of the genetic background on cognitive performances of TG2576 mice. Behav Brain Res 191:104–110. doi: 10.1016/j.bbr.2008.03.017

Latta CH, Brothers HM, Wilcock DM (2015) Neuroinflammation in Alzheimer's disease; A source of heterogeneity and target for personalized therapy. Neuroscience 302:103–111. doi: 10.1016/j.neuroscience.2014.09.061

Lee H, Castellani R, Zhu X, et al (2005) Amyloid-beta in Alzheimer's disease: The horse or the cart? Pathogenic or protective? Int J Exp Pathol 86:133–138. doi: 10.1111/j.0959-9673.2005.00429.x

Leissring MA, Farris W, Chang AY, et al (2003) Enhanced proteolysis of β-Amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. Neuron 40:1087–1093. doi: 10.1016/s0896-6273(03)00787-6

Lesné S, Ming TK, Kotilinek L, et al (2006) A specific amyloid-b protein assembly in the brain impairs memory. Nature 440:352–357. doi: 10.1038/nature04533

Lesné SE, Sherman MA, Grant M, et al (2013) Brain amyloid-β oligomers in ageing and Alzheimer's disease. Brain 136:1383–1398. doi: 10.1093/brain/awt062

Levison SW, Jiang FJ, Stoltzfus OK, Ducceschi MH (2000) IL-6-type cytokines enhance epidermal growth factor-stimulated astrocyte proliferation. Glia 32:328–337. doi: 10.1002/1098-1136(200012)32:3<328::AID-GLIA110>3.0.CO;2-7

Li A, Katafuchi T, Oda S, et al (1997) Interleukin-6 inhibits long-term potentiation in rat hippocampal slices. Brain Res 748:30–38

Li G, Klein RL, Matheny M, et al (2002) Induction of uncoupling protein 1 by central interleukin-6 gene delivery is dependent on sympathetic innervation of brown adipose tissue and underlies one mechanism of body weight reduction in rats. Neuroscience 115:879–889. doi: 10.1016/S0306-4522(02)00447-5

Lindwall G, Cole RD (1984) Phosphorylation affects the ability of tau protein to promote microtubule assembly. 259:5301–5305

Lipp H, Wolfer D (1998) Genetically modified mice and cognition: Current opinion in neurobiology. 8:272–280

Liu B, Liao J, Rao X, et al (1998) Inhibition of Stat1-mediated gene activation by PIAS1. Proc Natl Acad Sci 95:10626–10631. doi: 10.1073/pnas.95.18.10626

Liu F, Shi J, Tanimukai H, et al (2009) Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. Brain 132:1820–1832. doi: 10.1093/brain/awp099

Livingston G, Sommerlad A, Orgeta V, et al (2017) Dementia prevention, intervention, and care. Lancet 390:2673–2734. doi: 10.1016/S0140-6736(17)31363-6

Lust JA, Donovan KA, Kline MP, et al (1992) Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. Cytokine 4:96–100. doi: 10.1016/1043-4666(92)90043-Q

Lüth HJ, Apelt J, Ihunwo AO, et al (2003) Degeneration of β-amyloid-associated cholinergic structures in transgenic APPSW mice. Brain Res 977:16–22. doi: 10.1016/S0006-8993(03)02658-1

Lütticken C, Wegenka UM, Yuan J, et al (1994) Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science (80-) 263:89–92. doi: 10.1126/science.8272872

Mackic JB, Stins M, Mccomb JG, et al (1998) Human blood-brain Barrier receptors for Alzheimer's Amyloid-beta 1-40. J Clin Invest 102:734–743

Manso Y, Carrasco J, Comes G, et al (2012a) Characterization of the role of metallothionein-3 in an animal model of Alzheimer's disease. Cell Mol Life Sci 69:3683–3700. doi: 10.1007/s00018-012-1047-9

Manso Y, Carrasco J, Comes G, et al (2012b) Characterization of the role of the antioxidant proteins metallothioneins 1 and 2 in an animal model of Alzheimer's disease. Cell Mol Life Sci 69:3665–3681. doi: 10.1007/s00018-012-1045-y

Manso Y, Carrasco J, Comes G, et al (2012c) Characterization of the role of metallothionein-3 in an animal model of Alzheimer's disease. Cell Mol Life Sci 69:3683–3700. doi: 10.1007/s00018-012-1047-9

Manso Y, Comes G, López-Ramos JC, et al (2016) Overexpression of Metallothionein-1 modulates the phenotype of the Tg2576 mouse model of Alzheimer's disease. J Alzheimer's Dis 51:81–95. doi: 10.3233/JAD-151025

Marchese M, Cowan D, Head E, et al (2014) Autoimmune manifestations in the 3xTg-AD model of Alzheimer's disease. J Alzheimer's Dis 39:191–210. doi: 10.1097/CCM.0b013e31823da96d.Hydrogen

Marz P, Cheng J-G, Gadient RA, et al (1998) Sympathetic neurons can produce and respond to interleukin 6. Proc Natl Acad Sci 95:3251–3256. doi: 10.1073/pnas.95.6.3251

März P, Heese K, Dimitriades-Schmutz B, et al (1999) Role of interleukin-6 and soluble IL-6 receptor in region-specific induction of astrocytic differentiation and neurotrophin expression. Glia 26:191–200. doi: 10.1002/(SICI)1098-1136(199905)26:3<191::AID-GLIA1>3.0.CO;2-#

Masters CL, Simms G, Weinman NA, et al (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 82:4245–4249. doi: 10.1073/pnas.82.12.4245

Mastrangelo MA, Bowers WJ (2008) Detailed immunohistochemical characterization of temporal and spatial progression of Alzheimer's disease-related pathologies in male triple-transgenic mice. BMC Neurosci 9:1–31. doi: 10.1186/1471-2202-9-81

Matthews V, Schuster B, Schütze S, et al (2003) Cellular cholesterol depletion triggers shedding of the human interleukin-6 receptor by ADAM10 and ADAM17 (TACE). J Biol Chem 278:38829–38839. doi: 10.1074/jbc.M210584200

Matthews VB, Allen TL, Risis S, et al (2010) Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. Diabetologia 53:2431–2441. doi: 10.1007/s00125-010-1865-y

Mattsson N, Andreasson U, Zetterberg H, et al (2017) Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol 74:557–566. doi: 10.1001/jamaneurol.2016.6117

May LT, Ghrayeb J, Santhanam U, et al (1988a) Synthesis and secretion of multiple forms of β2-interferon/B-cell differentiation factor 2/hepatocyte-stimulating factor by human fibroblasts and monocytes. J Biol Chem 263:7760–7766

May LT, Santhanam U, Tatter SB, et al (1988b) Phosphorylation of secreted forms of human beta2-interferon/hepatocyte stimulating factor/interleukin-6. Biochem Biophys Res Commun 152:1144–1150

Metsalu T, Vilo J (2015) ClustVis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res 43:W566–W570. doi: 10.1093/nar/gkv468

Michaelson DM (2014) APOE ε4: The most prevalent yet understudied risk factor for Alzheimer's disease. Alzheimer's Dement 10:861–868. doi: 10.1016/j.jalz.2014.06.015

Milner R, Campbell IL (2006) Increased expression of the β4 and α5 integrin subunits in cerebral blood vessels of transgenic mice chronically producing the pro-inflammatory cytokines IL-6 or IFN-α in the central nervous system. Mol Cell Neurosci 33:429–440. doi: 10.1016/j.mcn.2006.09.004

Min SW, Cho SH, Zhou Y, et al (2010) Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron 67:953–966. doi: 10.1016/j.neuron.2010.08.044

Mishra D, Richard JE, Maric I, et al (2019) Parabrachial interleukin-6 reduces body weight and food intake and increases thermogenesis to regulate energy metabolism. Cell Rep 26:3011-3026.e5. doi: 10.1016/j.celrep.2019.02.044

Mohamed-Ali V, Goodrick S, Rawesh A, et al (1997) Subcutaneous adipose tissue releases Interleukin-6, but not Tumor Necrosis Factor- alfa, in vivo. J Clin Endocrinol Metab 82:4196–4200

Mohlenhoff B, Chao LL, Buckley ST, et al (2014) Are hippocampal size differences in posttraumatic stress disorder mediated by sleep pathology? Alzheimer's Dement 10:S146–S154. doi: 10.1038/mp.2011.182.doi

Moreno H, Morfini G, Buitrago L, et al (2016) Tau pathology-mediated presynaptic dysfunction. Neuroscience 325:30–38. doi: 10.1016/j.neuroscience.2016.03.044

Morris RGM (1981) Spatial localization does not require the presence of local cues. Learn Motiv 12:239–260. doi: http://dx.doi.org/10.1016/0023-9690(81)90020-5

Mrak RE (2009) Neuropathology and the neuroinflammation idea. J Alzheimer's Dis 18:473–481. doi: 10.3233/JAD-2009-1158

Mülberg J, Schooltink H, Stoyan T, et al (1993) The soluble interleukin-6 receptor is generated by shedding. Eur J Immunol 23:473–480. doi: 10.1002/eji.1830230226

Müllberg J, Oberthür W, Lottspeich F, et al (1994) The soluble human IL-6 receptor. Mutational characterization of the proteolytic cleavage site. J Immunol

152:4958–4968

Müller UC, Deller T, Korte M (2017) Not just amyloid: Physiological functions of the amyloid precursor protein family. Nat Rev Neurosci 18:281–298. doi: 10.1038/nrn.2017.29

Murakami M, Hibi M, Nakagawa N, et al (1993) IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. Science (80-) 260:1808–1810

Murphy MP, LeVine III H (2010) Alzheimer`s Disease and the β-Amyloid Peptide. J Alzheimer's Dis 19:1–17. doi: 10.3233/JAD-2010-1221.Alzheimer

National Institute on Aging (2016) Alzheimer's Disease Fact Sheet. https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet. Accessed 4 Apr 2019

Navia B, Ferrer B, Giralt M, et al (2014) Interleukin-6 deletion in mice driven by aP2-Cre-ERT2 prevents against high-fat diet-induced gain weight and adiposity in female mice. Acta Physiol 211:585–596. doi: 10.1111/apha.12328

Niwa K, Kazama K, Younkin L, et al (2002a) Cerebrovascular autoregulation is profoundly impaired in mice overexpressing amyloid precursor protein. Am J Physiol Circ Physiol 283:H315–H323. doi: 10.1152/ajpheart.00022.2002

Niwa K, Kazama K, Younkin SG, et al (2002b) Alterations in cerebral blood flow and glucose utilization in mice overexpressing the amyloid precursor protein. Neurobiol Dis 9:61–68. doi: 10.1006/nbdi.2001.0460

O'Bryant SE, Mielke MM, Rissman RA, et al (2017) Blood-based biomarkers in Alzheimer disease: Current state of the science and a novel collaborative paradigm for advancing from discovery to clinic. Alzheimer's Dement 13:45–58. doi: 10.1016/j.jalz.2016.09.014

Oberg HH, Wesch D, Grüssel SS, et al (2006) Differential expression of CD126 and CD130 mediates different STAT-3 phosphorylation in CD4+CD25- and CD25high regulatory T cells. Int Immunol 18:555–563. doi: 10.1093/intimm/dxh396

Oddo S, Billings L, Kesslak JP, et al (2004) Aβ immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 43:321–332

Oddo S, Caccamo A, Kitazawa M, et al (2003a) Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging 24:1063–1070. doi: 10.1016/j.neurobiolaging.2003.08.012

Oddo S, Caccamo A, Shepherd JD, et al (2003b) Triple-transgenic model of Alzheimer's Disease with plaques and tangles: Intracellular Aβ and synaptic dysfunction. Neuron 39:409–421. doi: 10.1016/S0896-6273(03)00434-3

Ödemis V, Moepps B, Gierschik P, Engele J (2002) Interleukin-6 and cAMP induce stromal cell-derived factor-1 chemotaxis in astroglia by up-regulating CXCR4 cell surface expression. J Biol Chem 277:39801–39808. doi: 10.1074/jbc.M200472200

Ognibene E, Middei S, Daniele S, et al (2005) Aspects of spatial memory and behavioral disinhibition in Tg2576 transgenic mice as a model of Alzheimer's disease. Behav Brain Res 156:225–232. doi: 10.1016/j.bbr.2004.05.028

Oh JW, Van Wagoner NJ, Rose-John S, Benveniste EN (1998) Role of IL-6 and the soluble IL-6 receptor in inhibition of VCAM-1 gene expression. J Immunol 161:4992–9

Ohsawa I, Takamura C, Morimoto T, et al (1999) Amino-terminal region of secreted form of amyloid precursor protein stimulates proliferation of neural stem cells. Eur J Neurosci 11:1907–1913. doi: 10.1046/j.1460-9568.1999.00601.x

Ohsawa K, Imai Y, Sasaki Y, Kohsaka S (2004) Microglia/macrophage-specific protein Iba1 binds to fimbrin and enhances its actin-bundling activity. J Neurochem 88:844–856. doi: 10.1046/j.1471-4159.2003.02213.x

Okada S, Nakamura M, Katoh H, et al (2006) Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat Med 12:829–834. doi: 10.1038/nm1425

Orihuela R, McPherson CA, Harry GJ (2016) Microglial M1/M2 polarization and metabolic states. Br J Pharmacol 173:649–665. doi: 10.1111/bph.13139

Ostojic J, Elfgren C, Passant U, et al (2004) The tau R406W mutation causes progressive presenile dementia with bitemporal atrophy. Dement Geriatr Cogn Disord 17:298–301. doi: 10.1159/000077158

Pang Y, Fan LW, Zheng B, et al (2006) Role of interleukin-6 in lipopolysaccharide-induced brain injury and behavioral dysfunction in neonatal rats. Neuroscience 141:745–755. doi: 10.1016/j.neuroscience.2006.04.007

Paonessa G, Graziani R, De Serio A, et al (1995) Two distinct and independent sites on IL-6 trigger gp130 dimer formation and signalling. EMBO J 14:1942–1951

Pasquarelli N, Voehringer P, Henke J, Ferger B (2017) Effect of a change in housing conditions on body weight, behavior and brain neurotransmitters in male C57BL/6J mice. Behav Brain Res 333:35–42. doi: 10.1016/j.bbr.2017.06.018

Pellow S, Chopin P, File SE, Briely M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167. doi: http://dx.doi.org/10.1016/0165-0270(85)90031-7

Penkowa M, Molinero A, Carrasco J, Hidalgo J (2001) Interleukin-6 deficiency reduces the brain inflammatory response and increases oxidative stress and neurodegeneration after kainic acid-induced seizures. Neuroscience 102:805–818. doi: 10.1016/S0306-4522(00)00515-7

Phinney AL, Drisaldi B, Schmidt SD, et al (2003) In vivo reduction of amyloid-beta by a mutant copper transporter. Proc Natl Acad Sci 100:14193–14198. doi: 10.1073/pnas.2332851100

Pogue AI, Cui JG, Li YY, et al (2010) Micro RNA-125b (miRNA-125b) function in astrogliosis and glial cell proliferation. Neurosci Lett 476:18–22. doi: 10.1016/j.neulet.2010.03.054

Poli V, Balena R, Fattori E, et al (1994) lnterleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J 13:1189–1196

Poupart P, Vandenabeelel P, Cayphas S, et al (1987) B cell growth modulating and differentiating activity of recombinant human 26-kd protein (BSF2, HuIFN-beta2, HPGE). EMBO J 6:1219–1224

Profenno LA, Porsteinsson AP, Faraone S V. (2010) Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. Biol Psychiatry 67:505–512. doi: 10.1016/j.biopsych.2009.02.013

Qiu Z, Gruol DL (2003) Interleukin-6, β-amyloid peptide and NMDA interactions in rat cortical neurons. J Neuroimmunol 139:51–57. doi: 10.1016/S0165-5728(03)00158-9

Quintana A, Erta M, Ferrer B, et al (2013) Astrocyte-specific deficiency of interleukin-6 and its receptor reveal specific roles in survival, body weight and behavior. Brain Behav Immun 27:162–173. doi: 10.1016/j.bbi.2012.10.011

Quintana A, Molinero A, Borup R, et al (2008) Effect of astrocyte-targeted production of IL-6 on traumatic brain injury and its impact on the cortical transcriptome. Dev Neurobiol 68:195–208. doi: 10.1002/dneu

Quintanilla RA, Orellana DI, González-Billault C, Maccioni RB (2004) Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. Exp Cell Res 295:245–257. doi: 10.1016/j.yexcr.2004.01.002

Rabe B, Chalaris A, May U, et al (2008) Transgenic blockade of interleukin 6 transsignaling abrogates inflammation. Blood 111:1021–1028. doi: 10.1182/blood-2007-07-102137

Rademakers R, Cruts M, Sleegers K, et al (2005) Linkage and association studies identify a novel locus for Alzheimer Disease at 7q36 in a dutch population-based sample. Am J Hum Genet 77:643–652. doi: 10.1086/491749

Rae EA, Brown RE (2015) The problem of genotype and sex differences in life expectancy in transgenic AD mice. Neurosci Biobehav Rev 57:238–251. doi: 10.1016/j.neubiorev.2015.09.002

Ravaglia G, Paola F, Maioli F, et al (2006) Interleukin-1β and interleukin-6 gene polymorphisms as risk factors for AD: A prospective study. Exp Gerontol 41:85–92. doi: 10.1016/j.exger.2005.10.005

Riekkinen PJ, Sirvio J, Aaltonen M, Riekkinen P (1990) Effects of concurrent manipulations of nicotinic and muscarinic receptors on spatial and passive avoidance learning. Brain Res 37:405–410

Ring S, Weyer SW, Kilian SB, et al (2007) The secreted beta-amyloid precursor protein ectodomain APPs is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. J Neurosci 27:7817–7826. doi: 10.1523/JNEUROSCI.1026-07.2007

Ringheim GE, Szczepanik AM, Petko W, et al (2002) Enhancement of beta-amyloid precursor protein transcription and expression by the soluble interleukin-6 receptor/interleukin-6 complex. Mol Brain Res 55:35–44. doi: 10.1016/s0169-328x(97)00356-2

Rodríguez-Martín T, Cuchillo-Ibáñez I, Noble W, et al (2013) Tau phosphorylation affects its axonal transport and degradation. Neurobiol Aging 34:2146–2157. doi: 10.1016/j.neurobiolaging.2013.03.015

Rothaug M, Becker-Pauly C, Rose-John S (2016) The role of interleukin-6 signaling

in nervous tissue. Biochim Biophys Acta 1863:1218–1227. doi: 10.1016/j.bbamcr.2016.03.018

Rott O, Tontsch U, Fleischer B, Cash E (1993) Interleukin-6 production in "normal" and HTLV-1 tax-expressing brain-specific endothelial cells. Eur J Immunol 23:1987–1991

Roytblat L, Rachinsky M, Fisher A, et al (2000) Raised interleukin-6 levels in obese patients. Obes Res 8:673–5. doi: 10.1038/oby.2000.86

Saavedra L, Mohamed A, Ma V, et al (2007) Internalization of beta-amyloid peptide by primary neurons in the absence of Apolipoprotein E. J Biol Chem Publ JBC Pap Press 282:35722–35732. doi: 10.1074/jbc.M701823200

Sadagurski M, Norquay L, Farhang J, et al (2010) Human IL6 enhances leptin action in mice. Diabetologia 53:525–535. doi: 10.1007/s00125-009-1580-8

Samland H, Huitron-Resendiz S, Masliah E, et al (2003) Profound increase in sensitivity to glutamatergic- but not cholinergic agonist-induced seizures in transgenic mice with astrocyte production of IL-6. J Neurosci Res 73:176–187. doi: 10.1002/jnr.10635

Schaper F, Rose-John S (2015) Interleukin-6: Biology, signaling and strategies of blockade. Cytokine Growth Factor Rev 26:475–487. doi: 10.1016/j.cytogfr.2015.07.004

Schéle E, Benrick A, Grahnemo L, et al (2013) Inter-relation between Interleukin (IL)-1, IL-6 and body fat regulating circuits of the hypothalamic arcuate nucleus. J Neuroendocrinol 25:580–589. doi: 10.1111/jne.12033

Schéle E, Fekete C, Egri P, et al (2012) Interleukin-6 receptor α is co-localised with melanin-concentrating hormone in human and mouse hypothalamus. J Neuroendocrinol 24:930–943. doi: 10.1111/j.1365-2826.2012.02286.x

Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S (2011) The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta - Mol Cell Res 1813:878–888. doi: 10.1016/j.bbamcr.2011.01.034

Schemmert S, Schartmann E, Zafiu C, et al (2019) Aβ oligomer elimination restores cognition in transgenic Alzheimer's mice with full-blown pathology. Mol Neurobiol 56:2211–2223. doi: 10.1007/s12035-018-1209-3

Schiemann WP, Bartoe JL, Nathanson NM (1997) Box 3-independent signaling

mechanisms are involved in Leukemia Inhibitory Factor Receptor α- and gp130-mediated stimulation of Mitogen-activated Protein Kinase. J Biol Chem 272:16631–16636. doi: 10.1074/jbc.272.26.16631

Schmitz J, Weissenbach M, Haan S, et al (2000) SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130. J Biol Chem 275:12848–12856. doi: 10.1074/jbc.275.17.12848

Schöbitz B, De Kloet ER, Sutanto W, Holsboer F (1993) Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. Eur J Neurosci 5:1426–1435. doi: 10.1016/0304-3940(92)90046-a

Sehgal PB, Zilberstein A, Ruggieri RM, et al (1986) Human chromosome 7 carries the beta 2 interferon gene. Proc Natl Acad Sci 83:5219–5222. doi: 10.1073/pnas.83.14.5219

Seibenhener ML, Wooten MC (2015) Use of the Open Field maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp 1–6. doi: 10.3791/52434

Selkoe DJ, Podlisny MB, Joachim CL, et al (1988) Beta-amyloid precursor protein of Alzheimer disease occurs as 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues. Proc Natl Acad Sci 85:7341–7345. doi: 10.1073/pnas.85.19.7341

Selmaj KW, Farooq M, Norton WT, et al (1990) Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor. J Immunol 144:129–135

Sethi MK, Zaia J (2017) Extracellular matrix proteomics in schizophrenia and Alzheimer's disease. Anal Bioanal Chem 409:379–394. doi: 10.1007/s00216-016-9900-6

Sherwin JRA, Smith SK, Wilson A, Sharkey AM (2002) Soluble gp130 is up-regulated in the implantation window and shows altered secretion in patients with primary unexplained infertility. J Clin Endocrinol Metab 87:3953–3960. doi: 10.1210/jcem.87.8.8766

Shirazi R, Palsdottir V, Collander J, et al (2013) Glucagon-like peptide 1 receptor induced suppression of food intake, and body weight is mediated by central IL-1 and IL-6. Proc Natl Acad Sci 110:16199–16204. doi: 10.1073/pnas.1306799110

Shrikant P, Weber E, Jilling T, Benveniste EN (1995) Intercellular adhesion molecule-1 gene expression by glial cells. Differential mechanisms of inhibition

by IL-10 and IL-6. J Immunol 155:1489–1501

Smith M a, Hirai K, Hsiao K, et al (1998) Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. J Neurochem 70:2212–2215. doi: 10.1046/j.1471-4159.1998.70052212.x

Soba P, Eggert S, Wagner K, et al (2005) Homo- and heterodimerization of APP family members promotes intercellular adhesion. EMBO J 24:3624–3634. doi: 10.1038/sj.emboj.7600824

Sofroniew M V. (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638–647. doi: 10.1016/j.tins.2009.08.002

Somers W, Stahl M, Seehra JS (1997) 1.9 Å crystal structure of interleukin 6: Implications for a novel mode of receptor dimerization and signaling. EMBO J 16:989–997. doi: 10.1093/emboj/16.5.989

Spindler A, Renvall M, Nichols J, Ramsdell J (1996) Nutritional status of patients with Alzheimer's disease: a 1-year study. J Am Diet Assoc 96:1013–1018. doi: 10.1016/S0002-8223(96)00270-2

Spooren A, Kolmus K, Laureys G, et al (2011) Interleukin-6, a mental cytokine. Brain Res Rev 67:157–183. doi: 10.1016/j.brainresrev.2011.01.002

Stahl N, Boulton TG, Farruggella T, et al (1994) Association and activation of Jak-Tyk Kinases by CNTF-LIF-OSM-IL-6 beta receptor components. Science (80-) 263:92–95

Stahl N, Farruggella T, Boulton T, et al (1995) Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. Science (80-) 267:1349–1354. doi: 10.1126/science.7871433

Steen RG, Mull C, Mcclure R, et al (2006) Brain volume in first-episode schizophrenia : Systematic review and meta-analysis of magnetic resonance imaging studies. Br J Psychiatry 188:510–518. doi: 10.1192/bjp.188.6.510

Stenlöf K, Wernstedt I, Fjällman T, et al (2003) Interleukin-6 levels in the central nervous system are negatively correlated with fat mass in overweight/obese subjects. J Clin Endocrinol Metab 88:4379–4383. doi: 10.1210/jc.2002-021733

Sterniczuk R, Antle MC, Laferla FM, Dyck RH (2010) Characterization of the 3xTg-AD mouse model of Alzheimer's disease: Part 2. Behavioral and cognitive changes. Brain Res 1348:149–155. doi: 10.1016/j.brainres.2010.06.011

Strittmatter WJ, Saunders AM, Schmechel D, et al (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A 90:1977–81

Sukoff Rizzo SJ, Neal SJ, Hughes ZA, et al (2012) Evidence for sustained elevation of IL-6 in the CNS as a key contributor of depressive-like phenotypes. Transl Psychiatry 2:e199-12. doi: 10.1038/tp.2012.120

Sun BL, Li WW, Zhu C, et al (2018) Clinical research on Alzheimer's Disease: progress and perspectives. Neurosci Bull 34:1111–1118. doi: 10.1007/s12264-018-0249-z

Swiergiel AH, Dunn AJ (2006) Feeding, exploratory, anxiety- and depression-related behaviors are not altered in interleukin-6-deficient male mice. Behav Brain Res 171:94–108. doi: 10.1038/mp.2011.182.doi

Taga T, Hibi M, Hirata Y, et al (1989) Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell 58:573–581

Takahashi-Tezuka M, Yoshida Y, Fukada T, et al (2015) Gab1 Acts as an adapter molecule linking the cytokine receptor gp130 to ERK mitogen-activated protein kinase. Mol Cell Biol 18:4109–4117. doi: 10.1128/mcb.18.7.4109

Takeda H, Tsuji M, Matsumiya T (1998) Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol 350:21–29. doi: 10.1016/S0014-2999(98)00223-4

Talbot C, Wang H-Y, Kazi H, et al (2012) Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and Cogniti. J Clin Invest 122:1316–1338. doi: 10.1172/JCI59903DS1

Tan MS, Yu JT, Tan L (2013) Bridging integrator 1 (BIN1): Form, function, and Alzheimer's disease. Trends Mol Med 19:594–603. doi: 10.1016/j.molmed.2013.06.004

Tanaka Y, Tanaka N, Saeki Y, et al (2008) c-Cbl-dependent monoubiquitination and lysosomal degradation of gp130. Mol Cell Biol 28:4805–4818. doi: 10.1128/mcb.01784-07

Tancredi V, D'Antuono M, Cafè C, et al (2000) The inhibitory effects of interleukin-6 on synaptic plasticity in the rat hippocampus are associated with an inhibition of mitogen-activated protein kinase ERK. J Neurochem 75:634–643. doi:

10.1046/j.1471-4159.2000.0750634.x

Tang Y, Le W (2016) Differential Roles of M1 and M2 microglia in neurodegenerative diseases. Mol Neurobiol 53:1181–1194. doi: 10.1007/s12035-014-9070-5

Tanzi RE (2012) The genetics of Alzheimer disease. Cold Spring Harb Perspect Med 2:a006296. doi: 10.1101/cshperspect.a006296

Thiel S, Sommer U, Kortylewski M, et al (2000) Termination of IL-6-induced STAT activation is independent of receptor internalization but requires de novo protein synthesis. FEBS Lett 470:15–19. doi: 10.1016/S0014-5793(00)01276-X

Thinakaran G, Koo EH (2008) Amyloid precursor protein trafficking, processing, and function. J Biol Chem 283:29615–29619. doi: 10.1074/jbc.R800019200

Timper K, Denson JL, Steculorum SM, et al (2017) IL-6 improves energy and glucose homeostasis in obesity via enhanced central IL-6 trans-signaling. Cell Rep 19:267–280. doi: 10.1016/j.celrep.2017.03.043

Toda T, Noda Y, Ito G, et al (2011) Presenilin-2 mutation causes early amyloid accumulation and memory impairment in a transgenic mouse model of Alzheimer's disease. J Biomed Biotechnol 2011:1–12. doi: 10.1155/2011/617974

Torres-Lista V, De La Fuente M, Giménez-Llort L (2017) Survival curves and behavioral profiles of female 3xTg-AD mice surviving to 18-months of age as compared to mice with normal aging. J Alzheimer's Dis Reports 1:47–57. doi: 10.3233/ADR-170011

Trautwein C, Rakemann T, Niehof M, et al (1996) Acute-phase response factor, increased binding, and target gene transcription during liver regeneration. Gastroenterology 110:1854–1862. doi: 10.1053/gast.1996.v110.pm8964411

Valle ML, Dworshak J, Sharma A, et al (2019) Inhibition of interleukin-6 trans-signaling prevents inflammation and endothelial barrier disruption in retinal endothelial cells. Exp Eye Res 178:27–36. doi: 10.1016/j.exer.2018.09.009

Van Dam D, De Deyn PP (2011) Animal models in the drug discovery pipeline for Alzheimer's disease. Br J Pharmacol 164:1285–1300. doi: 10.1111/j.1476-5381.2011.01299.x

von Koch CS, Zheng H, Chen H, et al (1997) Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. Neurobiol Aging

18:661–669. doi: 10.1016/s0197-4580(97)00151-6

Vorhees C V, Williams MT (2006) Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1:848–858. doi: 10.1038/nprot.2006.116

Vozarova B, Weyer C, Hanson K, et al (2001) Circulating Interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes Res 9:414–417. doi: 10.1038/oby.2001.54

Vukic V, Callaghan D, Walker D, et al (2009) Expression of inflammatory genes induced by beta-amyloid peptides in human brain endothelial cells and in Alzheimer's brain is mediated by the JNK-AP1 signaling pathway. Neurobiol Dis 34:95–106. doi: 10.1016/j.nbd.2008.12.007

Walf AA, Frye CA (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2:322–328. doi: 10.1038/nprot.2007.44

Wallace L, Howlett S, Reid A, et al (2013) Frailty in relation to neuropathological markers of Alzheimer's disease: Evidence from a transgenic mouse model. Alzheimer's Dement 9:P500. doi: 10.1016/j.jalz.2013.05.1035

Wallenius K, Wallenius V, Sunter D, et al (2002a) Intracerebroventricular interleukin-6 treatment decreases body fat in rats. Biochem Biophys Res Commun 293:560–565. doi: 10.1016/S0006-291X(02)00230-9

Wallenius V, Wallenius K, Ahrén B, et al (2002b) Interleukin-6-deficient mice develop mature-onset obesity. Nat Med 8:75–79. doi: 10.1038/nm0102-75

Walsh RN, Cummins RA (1976) The Open-Field Test: a critical review. Psychol Bull 83:482–504. doi: 10.1037/0033-2909.83.3.482

Wang J-Z, Grundke-Iqbal I, Iqbal K (1996) Glycosylation of microtubule-associated protein tau: An abnormal posttranslational modification in Alzheimer's disease. Nat Med 2:871–875

Ward LD, Howlettn J, Discolo G, et al (1994) High affinity interleukin-6 receptor is a hexameric complex consisting of two molecules each of interleukin-6, interleukin-6 receptor, and gp130. J Biol Chem 269:23286–23289

Wasco W, Gurubhagavatula S, Paradis MD, et al (1993) Isolation and characterization of APLP2 encoding a homologue of the Alzheimer's associated amyloid beta protein precursor. Nat Genet 5:95–100. doi: 10.1038/ng0293-165

Watanabe T, Yamagata N, Takasaki K, et al (2009) Decreased acetylcholine release is correlated to memory impairment in the Tg2576 transgenic mouse model of Alzheimer's disease. Brain Res 1249:222–228. doi: 10.1016/j.brainres.2008.10.029

Wenk GL, Mcgann-Gramling K, Hauss-Wegrzyniak B (2004) The presence of the APPswe mutation in mice does not increase the vulnerability of cholinergic basal forebrain neurons to neuroinflammation. Neuroscience 125:769–776. doi: 10.1016/j.neuroscience.2004.01.050

Westmark CJ, Westmark PR, Beard AM, et al (2008) Seizure susceptibility and mortality in mice that over-express amyloid precursor protein. Int J Clin Exp Pathol 1:157–68

Whitehead JC, Hildebrand BA, Sun M, et al (2014) A clinical frailty index in aging mice: Comparisons with frailty index data in humans. Journals Gerontol - Ser A Biol Sci Med Sci 69:621–632. doi: 10.1093/gerona/glt136

Woiciechowsky C, Schöning B, Stoltenburg-Didinger G, et al (2015) Brain-IL-1 β triggers astrogliosis through induction of IL-6 : Inhibition by propranolol and IL-10. Med Sci Monit 10:325–330

Wolf J, Rose-John S, Garbers C (2014) Interleukin-6 and its receptors: a highly regulated and dynamic system. Cytokine 70:11–20. doi: 10.1016/j.cyto.2014.05.024

Wood J, Wood P, Ryan R, et al (1993) Cytokine indices in Alzheimer's temporal cortex: no changes in mature IL-1 beta or IL-1RA but increases in the associated acute phase proteins IL-6, alfa2-macroglobulin and C-reactive protein. Brain Res 629:245–252. doi: 10.1016/0006-8993(93)91327-O

Wu Z, Yang B, Liu C, et al (2015) Long term Dantrolene treatment reduced intraneuronal amyloid in aged Alzheimer triple transgenic mice. Alzheimer Dis Assoc Disord 29:184–191. doi: 10.1007/s10741-014-9462-7.Natural

Wyss-coray T, Rogers J (2012) Inflammation in Alzheimer disease — A Brief review of the basic science and clinical literature. Cold Spring Harb Perspect Biol 1–24. doi: 10.1101/cshperspect.a006346

Xu F, Davis J, Miao J, et al (2005) Protease nexin-2/amyloid beta-protein precursor limits cerebral thrombosis. Proc Natl Acad Sci U S A 102:18135–18140

Xu F, Previti M Lou, Van Nostrand WE (2007) Increased severity of hemorrhage in

transgenic mice expressing cerebral protease nexin-2/amyloid β-protein precursor. Stroke 38:2598–2601. doi: 10.1161/STROKEAHA.106.480103

Xu Y, Kershaw NJ, Luo CS, et al (2010) Crystal structure of the entire ectodomain of gp130: Insights into the molecular assembly of the tall cytokine receptor complexes. J Biol Chem 285:21214–21218. doi: 10.1074/jbc.C110.129502

Yakar S, Nunez NP, Pennisi P, et al (2006) Increased tumor growth in mice with diet-induced obesity: Impact of ovarian hormones. Endocrinology 147:5826–5834. doi: 10.1210/en.2006-0311

Yamabe T, Dhir G, Cowan EP, et al (1994) Cytokine-gene expression in measles-infected adult human glial cells. J Neuroimmunol 49:171–179. doi: 10.1016/0165-5728(94)90193-7

Yamada M, Chiba T, Sasabe J, et al (2005) Implanted cannula-mediated repetitive administration of Aβ25-35 into the mouse cerebral ventricle effectively impairs spatial working memory. Behav Brain Res 164:139–146. doi: 10.1016/j.bbr.2005.03.026

Yamasaki K, Taga T, Hirata Y, et al (1988) Cloning and expression of the human Interleukin-6 (BSF-2/IFNbeta2) receptor. Science (80-) 241:825–828

Yasukawa K, Hirano T, Watanabe Y, et al (1987) Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene. EMBO J 6:2939–2945

Zhang J-M, An J (2009) Cytokines, Inflammation, Pain. Int Anesth Clin 69:482–489. doi: 10.1097/AIA.0b013e318034194e.Cytokines

Zhang X, Fu Z, Meng L, et al (2018) The early events that initiate β-amyloid aggregation in Alzheimer's Disease. Front Aging Neurosci 10:1–13. doi: 10.3389/fnagi.2018.00359

Zhang X, Yeung DCY, Karpisek M, et al (2008) Serum FGF21 levels are increased in obesity and are in humans. Diabetes 57:1246–1253. doi: 10.2337/db07-1476.Additional

Zhong Z, Wen Z, Darnell JEJ (1994) Stat3 : A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and Interleukin-6. Science (80-) 264:95–98

Zhuo JM, Prescott SL, Murray ME, et al (2007) Early discrimination reversal learning impairment and preserved spatial learning in a longitudinal study of

Tg2576 APPsw mice. Neurobiol Aging 28:1248–1257. doi: 10.1016/j.neurobiolaging.2006.05.034

Zolti M, Ben-Rafael Z, Meirom R, et al (1991) Cytokine involvement in oocytes and early embryos. Fertil Steril 56:265–272. doi: 10.1016/S0015-0282(16)54483-5

ACKNOWLEDGMENTS

I com no, arriba el moment dels agraïments, perquè una tesis "de veritat" més que esforç i patiment individual també requereix col·laboració i treball col·lectiu. Primer de tot volia agraïr a la gent que contribuïnt amb un donatiu ha fet possible tota aquesta feina… gràcies a la Marató de TV3, que fa possibles aquests projectes. Com ja sabeu, sempre m'ha semblat bonic dir que em paga la solidaritat dels ciutadans i ciutadanes!!

Al Juanma per haver-me donat aquesta oportunitat i haver-me ensenyat a nivell de ciència com d'escriptura (aquestes , darrere els whereas no se m'oblidaran mai!), no he sabut mai ben bé qui et va fer el "xivatazo" de que buscava doctorat, però se'ns dubte va cambiar el meu destí. Gràcies a l'Amalia i la Mercè per tota l'ajuda, com sent tan diferents sou capaces de formar un bon equip i acabar experiments en temps record. I moltes moltíssimes gràcies a la resta del grup, les persones amb qui he compartit més estones aquests últims anys, les glòries i les penes, i que han aguantat estoïcament els meus nervis i TOCs dels últims mesos, gràcies a la Paula, sempre serem Pili i Mili, valencianes les dos, però com si forem una de Mart i l'altra de Júpiter, gràcies per fer-me suport en tot i deixar de banda totes les competitivitats lletges que corren pel món científic. Gràcies a la Gemma, ja sabem que tens filles i són la teva prioritat però gràcies per fer de mare (no per edat eh!), ensenyar-me a relativitzar els problemas i posar una mica de seny quan he entrat en locura transitòria. També a la Olaya, ja et tenim ben lluny, però sabem que estàs al lloc on has d'estar, a NY on pots tenir tota la varietat de musicals i menjar que més t'agrada (anyore molt els teus pastissos!!!!!). Gràcies a totes vosaltres he après molt de ciència, però també de la vida. No em puc oblidar de les noves incorporacions del grup, la Carla i el Kevin. Carla, mini-jo i orgull Pompeu, gràcies per estar sempre disposada a ajudar quan faci falta (i disposar sempre d'alguna anécdota graciosa xD). Kevin, no hem pogut treballar tan de prop, però gràcies per ensenyar-me la jerga millenial i els bons ratos al bugui que lo flipas. I també gràcies als estudiants que han contribuït a la tesi: Atanas, Brenda i Alejandro. També al Javier, per resoldre dubtes encara que siga a canvi d'un tros de pastís.

Als del grup del costat, les "rates". Al mini-Gargamel Jesús per les seves lliçons de filosofia i literatura. A la Patri, pel seu entusiasme i bon humor. Molts ànims! Ho aconseguireu! A Xavi pels teus acudits (menys els verds!) i la teva gentilesa. Al maxi-Gargamel Humberto per les seves galetes de xocolata (que t'he robat, ho confesso... però de manera sophisticated!). A la Maria, gràcies per fer-me de mentora a l'any de màster, em vas ensenyar a fer "quasi" les primeres passes al laboratori (o almenys en conducta) i a treballar com toca (en silenci). Al Jordi, per explicar-me coses random d'informàtica i jo fer veure que t'entenc, gràcies per posar sempre el toc d'humor a l'hora de dinar (i a la resta del dia xD). Al Juanan, per la seva paciència, serenitat en mig de com tu diries any de caos y destrucción! A la Inés, per totes les ganes d'innovar i il·lusió que li poses a la recerca, no cambies! A l'Antonio i la Roser qui directa o indirectament em van obrir les portes per fer la tesis.

Als del grup de peixos. Camino, gràcies per tenir tanta energia i entusiasme per fer coses: muntanya, Apolo, Van Van... Juntament amb l'Olaya sou un pou sense fons de plans diversos per al cap de setmana, gràcies pels vermuts!!! Al Joan Carles, gràcies pels teus consells de disseny i maquetació, li has donat un toc de bon gust a aquest llibre! I a la resta de gent que forma part del departament, tothom heu contribuït a tancar aquesta etapa.

Als Bioguais, gràcies Jèssica, Helena, Sílvia, Pere, Paula i Marta. Gràcies per ser els meus amics a Barcelona. Per haver-nos mantingut, tot i les distàncies, per continuar com sempre. Per molts més escape rooms, viatges, Sant Joans i aventures vàries. Dintre i fora del món científic, m'heu aportat moltíssim durant aquests anys.

A les meues amigues del poble, les eternes, Mar, Marta, Andrea, Ali, Lidia i Laura. Gràcies per escoltar les meues històries quan torne de Catalunya, i moltes gràcies per estar sempre ahí. Per parlar per telèfon quan ho necessito i posar-nos al dia.

A Miquel, per cuidar-me i ser pacient aquest últim any. Hem patit la part més estressant de la tesis junts. Gràcies per mirar sempre la part bona de les coses, prioritzar les meves necessitats tesinenques, i fer-me desconnectar a base de correfocs, excursions i curiositats sobre la nostra benvolguda Barcelona.

A la meua família. Gràcies mare per preocupar-te tant, cridar incansablement per telèfon, tot i que responc de mala gana i gràcies pare per no fer-ho, per deixar-me anar a la meua i confiar en mi! Gràcies als dos sóc qui sóc i he arribat fins así! (I el que queda!)