Brain Health

Preclinical studies in Ldlr-/-.Leiden mice

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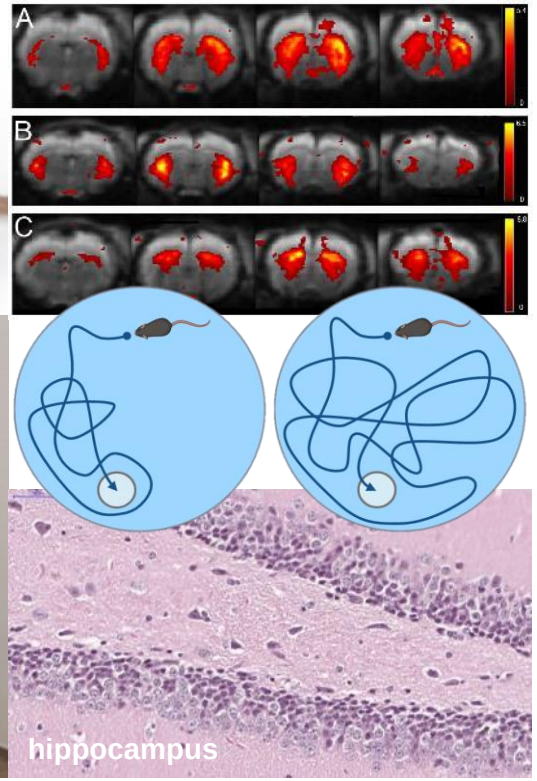
Model for Brain Health and Neuroinflammation (Aging)



- **Proprietary TNO strain: Ldlr-/-.Leiden mouse**
  - Translational for obesity, IR, NAFLD/NASH, CVD.
  - Human disease mechanisms and pathology
  - Mimics patient groups
    (Morrison, Hepatol Comm., 2018; Martinez-Arranz, Hepatology 2022)
- **Functional and molecular readouts: body & brain**
  - Behaviour & cognition tests during aging and/or obesity
  - Histopathology: morphology & protein expression and distribution
  - Brain: transcriptomics, lipids, oxylipins, neuroinflammation
- **MRI imaging with Radboudumc**
  - Brain structures (e.g. grey matter and white matter integrity)
  - Brain function (connectivity, cerebral blood flow)
  - Polarized light imaging

Neurodegeneration

Neurodegeneration

- Underlying disease process in all neurodegenerative diseases
- Loss of neuronal function – breakdown connectivity & communication: axonal damage, synaptic dysfunction/loss

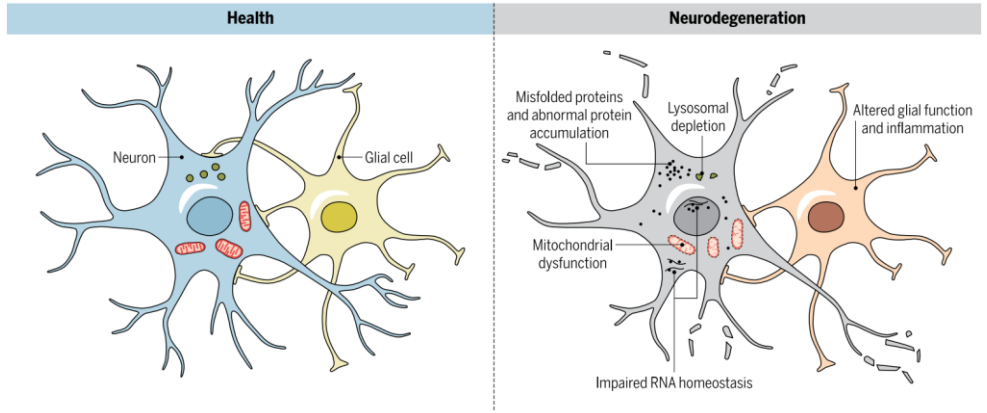
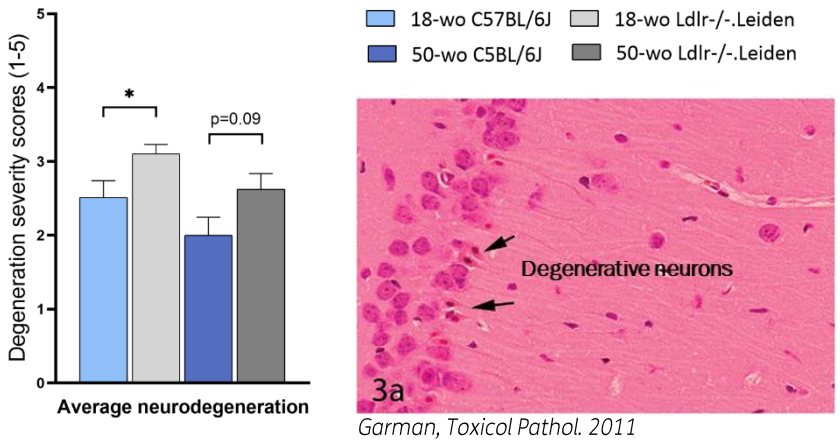


Fig. 1. Major cellular and molecular processes contributing to neurodegeneration. There are multiple processes that drive neurodegeneration as a result of specific genetic vulnerabilities or aging. Such processes include abnormally altered expression of some disease-driving RNAs and proteins, dysfunction of specific cellular organelles such as mitochondria or lysosomes, and neuroinflammation and altered responses of glia in the brain. Lysosomes and mitochondria are shown in green and pink, respectively. Abnormal protein accumulation and altered RNA-protein interactions are depicted as black dots.

Katnelson et al. Sci Transl Med. 2016

Histological analysis – comparison with conventional model (C57BL/6J)



Garman, Toxicol Pathol. 2011

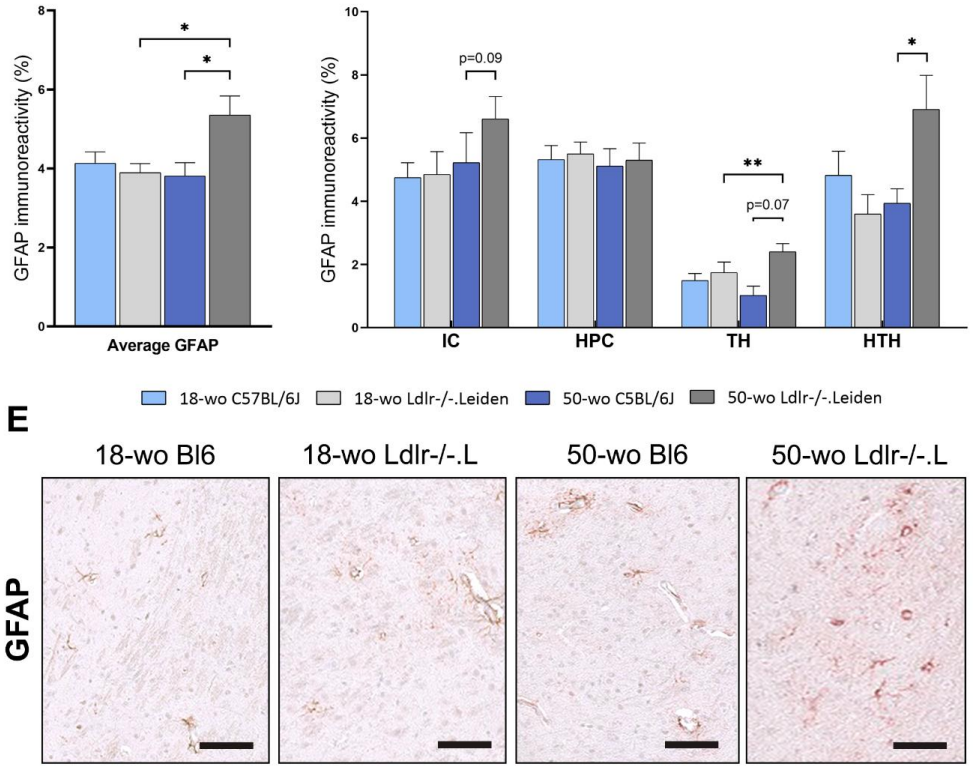
- Neurodegeneration more pronounced in Ldlr-/-.Leiden model than in conventional model.

Seidel et al. Front.Cell Neurosc. 2023

Age-related gliosis

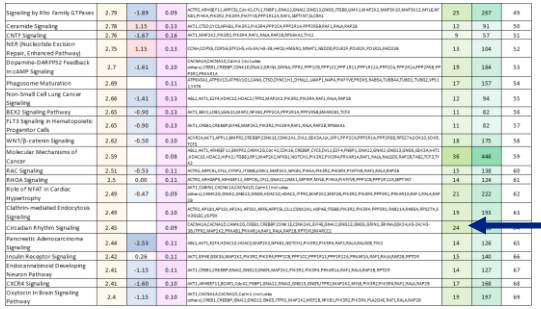
Gliosis

- Reactive change (proliferation) in glial cells in response to damage.
  - Glial cells: ‘helper cells’ of nervous system (homeostasis, myelination, BBB, supply neurons with nutrients, support & protect neurons)
  - Important pro-inflammatory mechanism in neurodegenerative diseases
- Astrogliosis: proliferation of astrocytes.
  - Index for neuronal damage.
  - GFAP: marker protein for astrocytes.
- Histological analysis – comparison with conventional model
  - Ldlr-/-.Leiden model shows age-related gliosis, that is not observed in conventional model

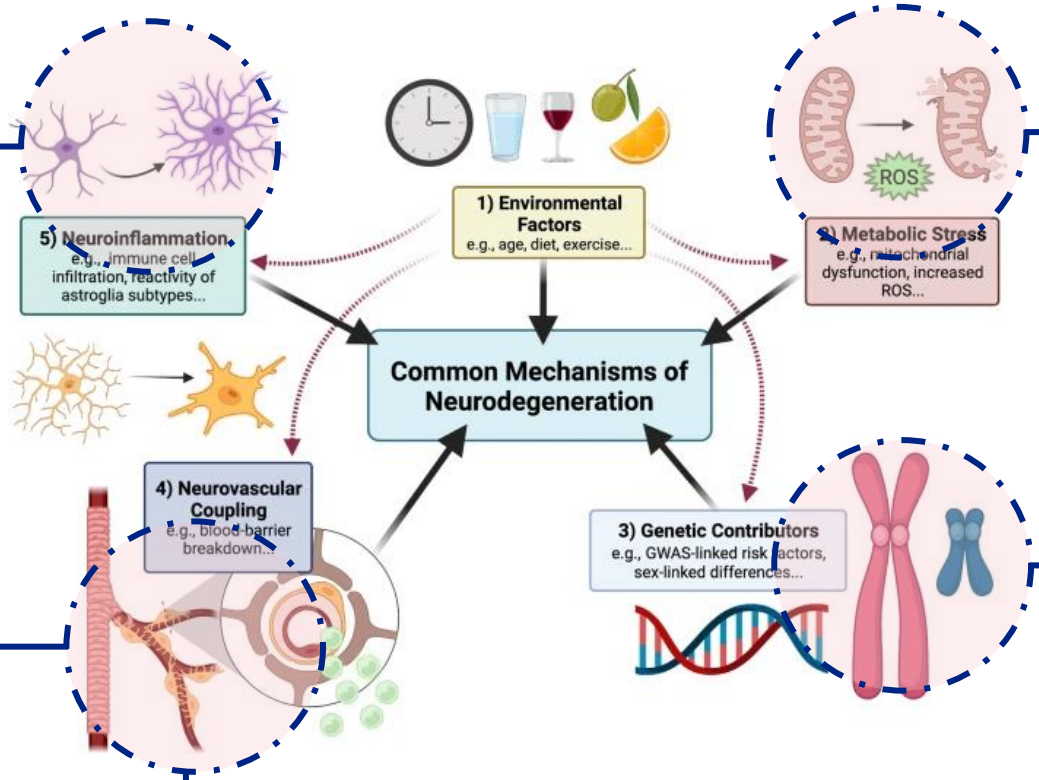


Seidel et al. Front.Cell Neurosc. 2023

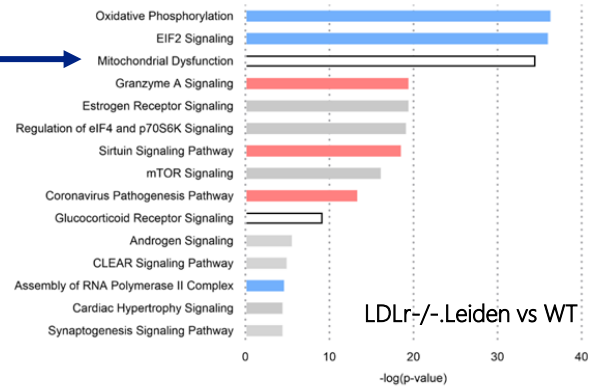
Mechanistic differences with conventional model



Inflammatory signalling in brain increased in Ldlr-/-.Leiden model



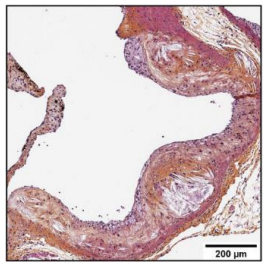
Increased metabolic oxidative stress in brain in Ldlr-/-.Leiden model
=> Neuroinflammation: altered cytokine protein expression & oxylipids



Whole genome sequencing (collaboration LUMC, ongoing)

Leiden University Medical Center

Vascular dysfunction is intrinsic to Ldlr-/-.Leiden model (atherosclerosis, increased blood pressure, CBF changes)

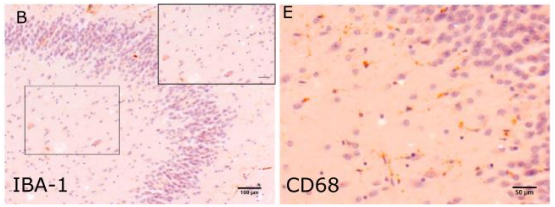


Established BBB dysfunction (MRI tracer studies, collaboration Radboudumc, ongoing)

Wareham et al. Molecular Neurodegeneration 2022

Susceptibility to obesity-induced neuroinflammation

- Neuroinflammation in obesity-associated brain dysfunction
  - Thought to play an important role in the cognitive deficits observed in obese subjects
- Classical marker for neuroinflammation: IBA1
  - Conventional neuroinflammation models show upregulation of IBA1
- Human data: <u>down</u>regulation of IBA1 in neuroinflammation/NDD:



Loss of IBA-1 expression in human obese subjects

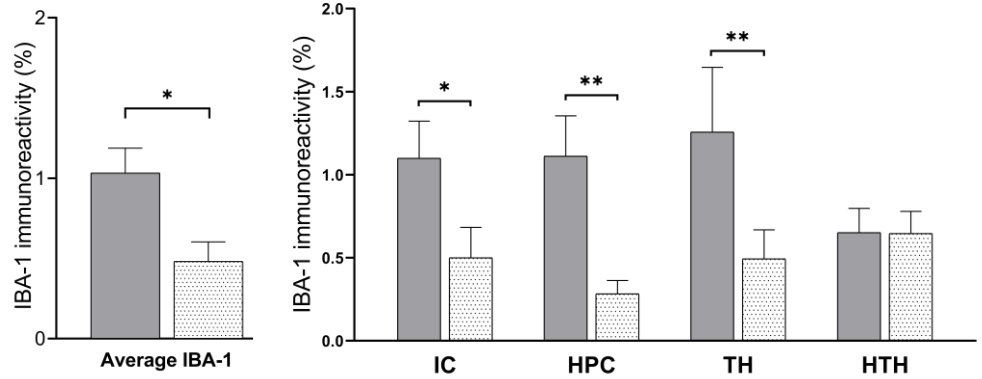
Lier et al. Brain Res. 2019

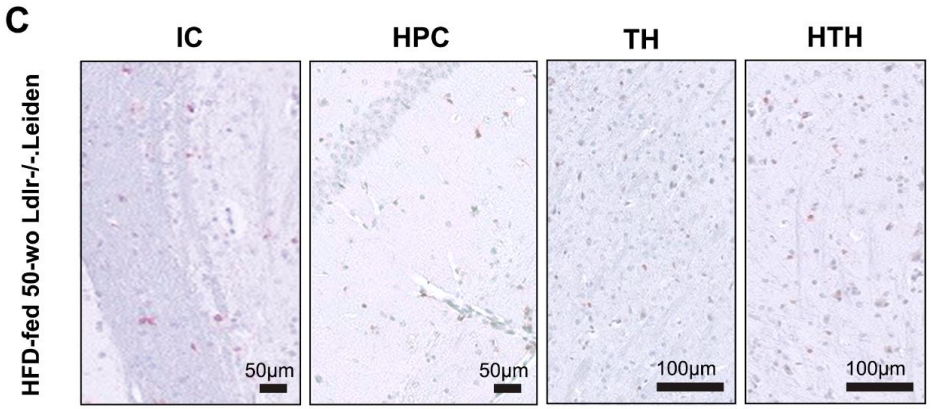
Table 4 Weighted logistic regression to analyse the relationship between microglial protein load (%) and dementia status in participants with and without Alzheimer's dementia

Microglia (load (%))	OR	95 % CI (OR)	P
Iba1	0.86	(0.82; 0.89)	<0.001
CD68	3.55	(1.93; 6.51)	<0.001
HLA-DR	1.06	(0.96; 1.18)	0.250
MSR-A	1.56	(1.11; 2.19)	0.010
CD64	1.21	(1.05; 1.39)	0.007

Negative correlation between IBA1 expression and Alzheimer's disease, positive correlation with other microglial markers

Minett et al. J Neuroinflammation 2016





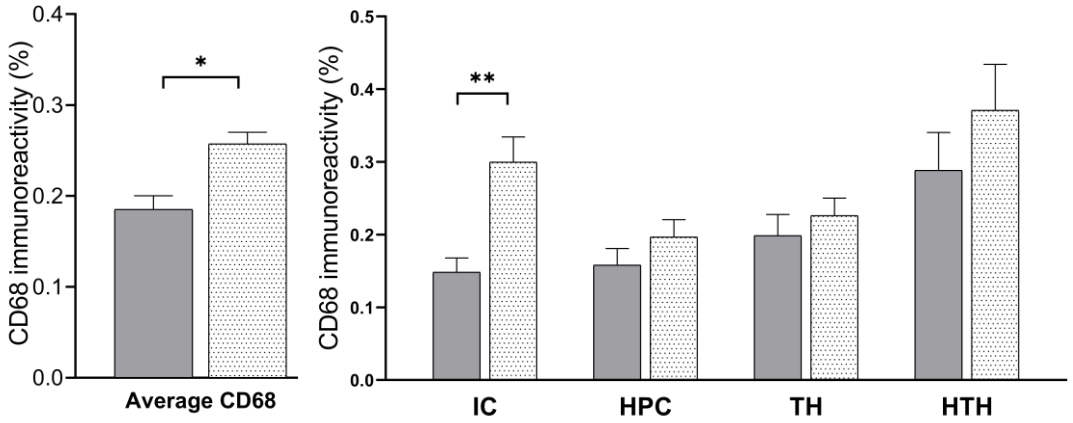
Seidel et al. Front.Cell Neurosc. 2023

Susceptibility to obesity-induced neuroinflammation

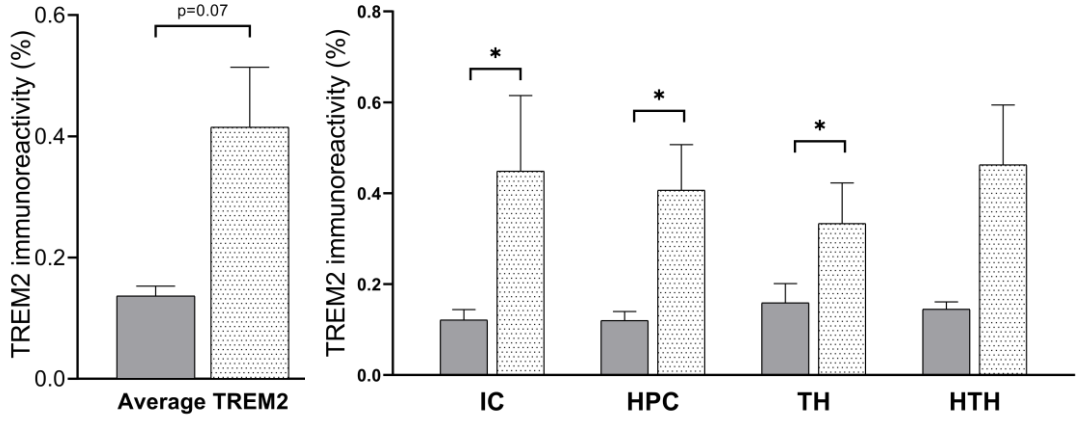
In line with human data, obesity-associated changes in immunophenotype in Ldlr-/-.Leiden model:

- Upregulation of other microglia markers (CD68 and TREM2): both linked to human neurodegenerative diseases

CD68 (phagocytic microglia)



TREM2 (microglia activation)



- Neuroinflammation is not simply upregulation of number of cells or a single marker => it is a change in immunotype (as also observed in human neurodegeneration).
- Ldlr-/-.Leiden model replicates microglial marker expression patterns observed in human neurodegenerative disease

Seidel et al. Front.Cell Neurosc. 2023

Obesity-induced neuroinflammation - cytokines

Effect of HFD feeding on chemokine and cytokine concentrations in cortex homogenates of 50 week-old Ldlr-/-Leiden mice

Cytokines	Chow		HFD	
	Mean	SEM	Mean	SEM
IL-17A/F	0,13	0,02	0,14	0,01
IL-27p28/IL-30	0,28	0,05	0,26	0,03
IL-33	38,82	11,22	78,50 p=0.07	13,68
IP-10	0,79	0,04	0,89	0,08
MCP-1	1,94	0,21	1,89	0,09
IFN-γ	0,00	0,00	0,00	0,00
IL-10	0,09	0,02	0,14 *	0,01
IL-1β	0,09	0,02	0,13 p=0.13	0,02
IL-2	0,03	0,00	0,03	0,00
IL-6	0,29	0,06	0,41*	0,02
KC/GRO	0,96	0,10	0,86	0,03
TNF-α	0,02	0,01	0,03 p=0.13	0,00

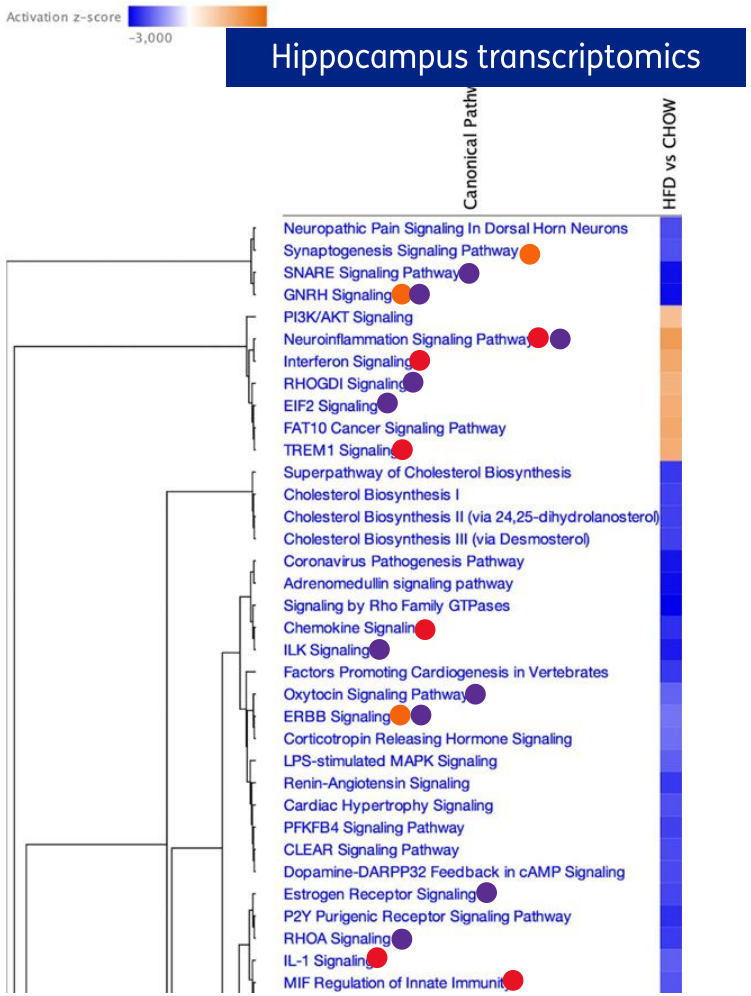
Concentrations are expressed as pg / mg protein.

- Neuroinflammation also observed on protein level: HFD induces inflammatory response as evidenced by cytokine profiling of cortex.
  - IL-33 ↑
  - IL-10 ↑
  - IL-1β ↑
  - IL-6 ↑
  - TNF-α ↑

HFD vs. Chow

Seidel et al. Front.Cell Neurosc. 2023

Obesity-induced neuroinflammation - mechanisms



- Activation of several neuroinflammatory pathways
  - In line with histological analysis: not all inflammatory pathways are upregulated, some also inactivated. → immunophenotypical change
- Deregulation of processes involved in neuron health/function:
  - E.g. axon ensheathment, neurotransmission, synaptic plasticity
- Many processes with described role in neurodegenerative disease

In conclusion:

- Ldlr-/-.Leiden model is more susceptible to neurodegeneration and associated gliosis than conventional C57BL/6J mouse model.
- HFD-induced obesity results in neuroinflammation characterised by immunophenotypic shift, similar to what is observed in humans.

Seidel et al. Front.Cell Neurosc. 2023

Publications Ldlr-/-.Leiden brain studies

	Intervention	Publication
SCFA (gut-derived mediators)	Butyrate	Arnoldussen et al. Int J Obesity 2017
	Propionate	Tengeler & Gart et al. FASEBJ 2020
(Breast) milk component	Milk fat globule membrane (MFGM)	Arnoldussen et al. Int J Obesity 2021
Muscle-targeted intervention	Exercise and branched-chain amino acids	Lohkamp et al. Nutrients 2023
Anti-inflammatory	Comparison Ldlr-/-.Leiden vs BL6 + anti-inflammatory treatment (anti-C5)	Seidel et al. Front.Cell Neurosc. 2023
Sex differences	Male vs female	Jacobs et al. Nutrients 2019

Brain Health Public Private Partnership Opportunity

Opportunity to join Brain Health PPP

Open for collaboration!

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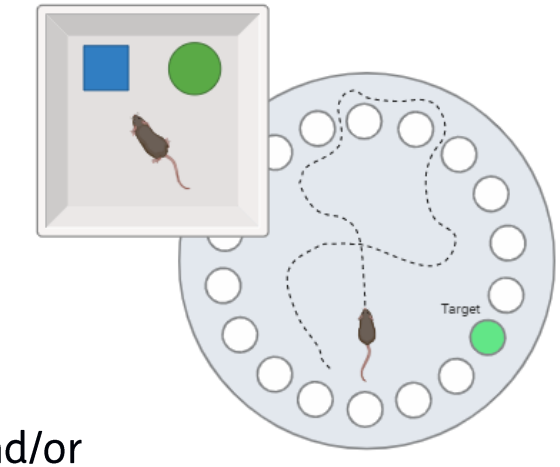
Mechanistic Readouts of Brain Health

+

Functional behavioural and cognitive tests

Neurodegeneration | Astrogliosis | Neuroinflammation

e.g. immunohistochemistry, cytokine profiling, gene expression (transcriptomics and pathway analysis), lipid- and oxylipin profiling



- Ldlr-/-.Leiden mice in the context of aging and/or dysmetabolism/obesity.
- Looking for partner(s) with interest to study treatment (prevention/intervention) to improve brain health.
- Publication of results.