UPPSALA UNIVERSITET

Epidemiology- and OMICs-based Development of a Test Battery for Developmental Neurotoxicity Induced by Endocrine Disruption

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Over 1000 chemicals are potential EDCs

Pesticides

Plastics

Flame retardants

Polybrominated

"An endocrine disruptor is an **exogenous substance** or mixture that **alters function(s) of the endocrine system and consequently causes adverse health effects** in an intact organism, or its progeny, or (sub)populations"

Paracetamol

Water repellents

Perfluorinated alkyl substances (PFAS)

Pr

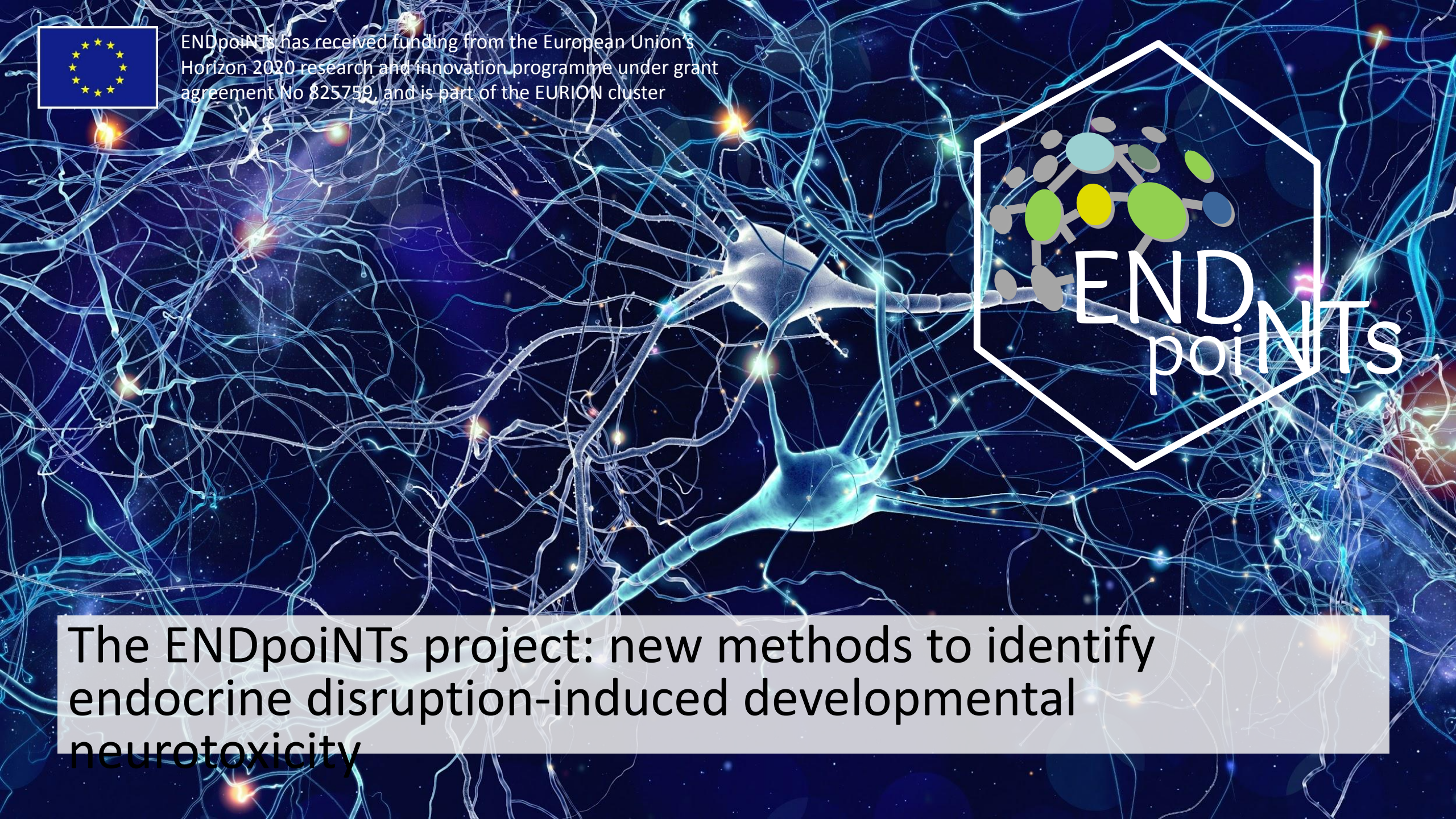
Parabens, nonylphenols

Estrogen system (ER)
Androgen system (AR)
Thyroid hormone system (ThR)

EDCs and brain development

Pesticides	Bisphenol A	Phthalates	Per- and polyfluoroalkyl substances (PFAS)
Lower mental developmental index, reduced IQ, attention deficits		Impaired attention in girls	Reduced IQ, attention deficits
	Aggression, hyperactivity, anxiety	Diminished masculine behavior in boys	Hyperactivity

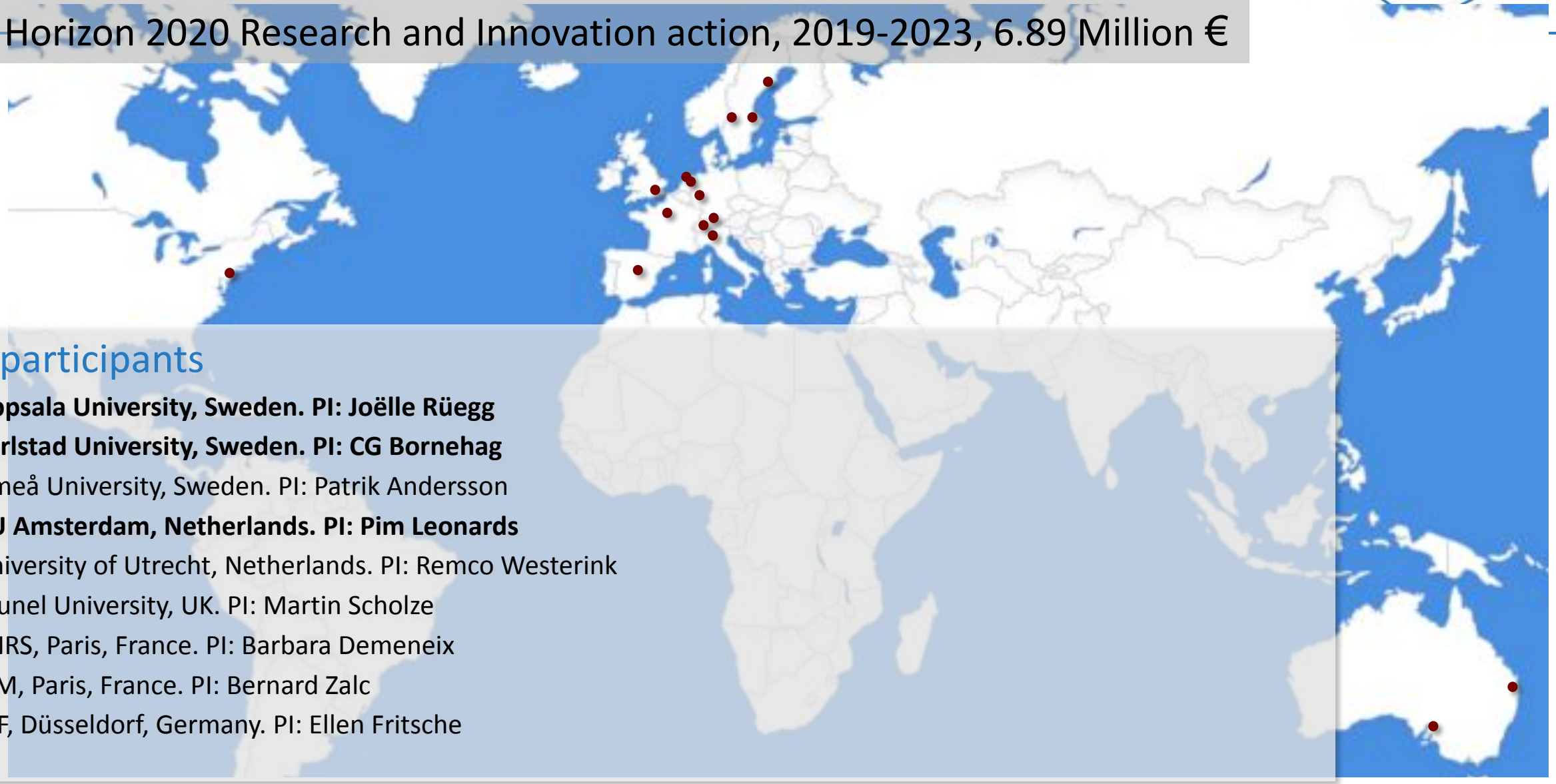
ENDpoiNTs has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825759, and is part of the EURION cluster



The ENDpoiNTs project: new methods to identify endocrine disruption-induced developmental neurotoxicity

ENDpoiNTs

Horizon 2020 Research and Innovation action, 2019-2023, 6.89 Million €



16 participants

- **Uppsala University, Sweden. PI: Joëlle Rüegg**
- **Karlstad University, Sweden. PI: CG Bornehag**
- Umeå University, Sweden. PI: Patrik Andersson
- **VU Amsterdam, Netherlands. PI: Pim Leonards**
- University of Utrecht, Netherlands. PI: Remco Westerink
- Brunel University, UK. PI: Martin Scholze
- CNRS, Paris, France. PI: Barbara Demeneix
- ICM, Paris, France. PI: Bernard Zalc
- IUF, Düsseldorf, Germany. PI: Ellen Fritsche

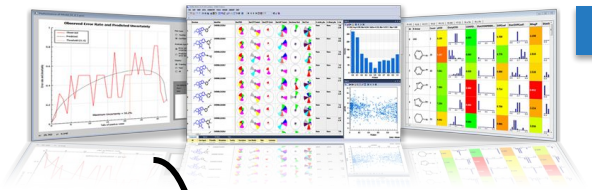
The scientific gap

"An endocrine disruptor is an **exogenous substance** or mixture that **alters function(s) of the endocrine system and consequently causes adverse health effects** in an intact organism, or its progeny, or (sub)populations"

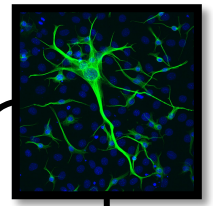
Basis for new methods

Chemical exposure → Endocrine mode of action → DNT-relevant molecular key events → DNT-relevant cellular key events → DNT outcomes

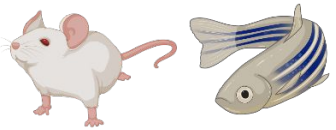
In silico



In vitro



In vivo

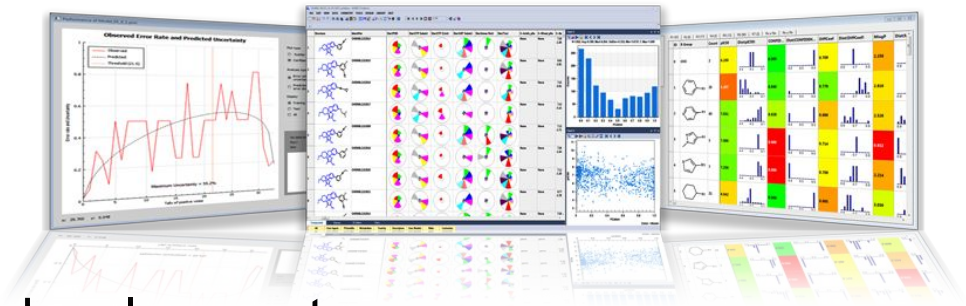


Hormonal involvement on neurodevelopmental processes

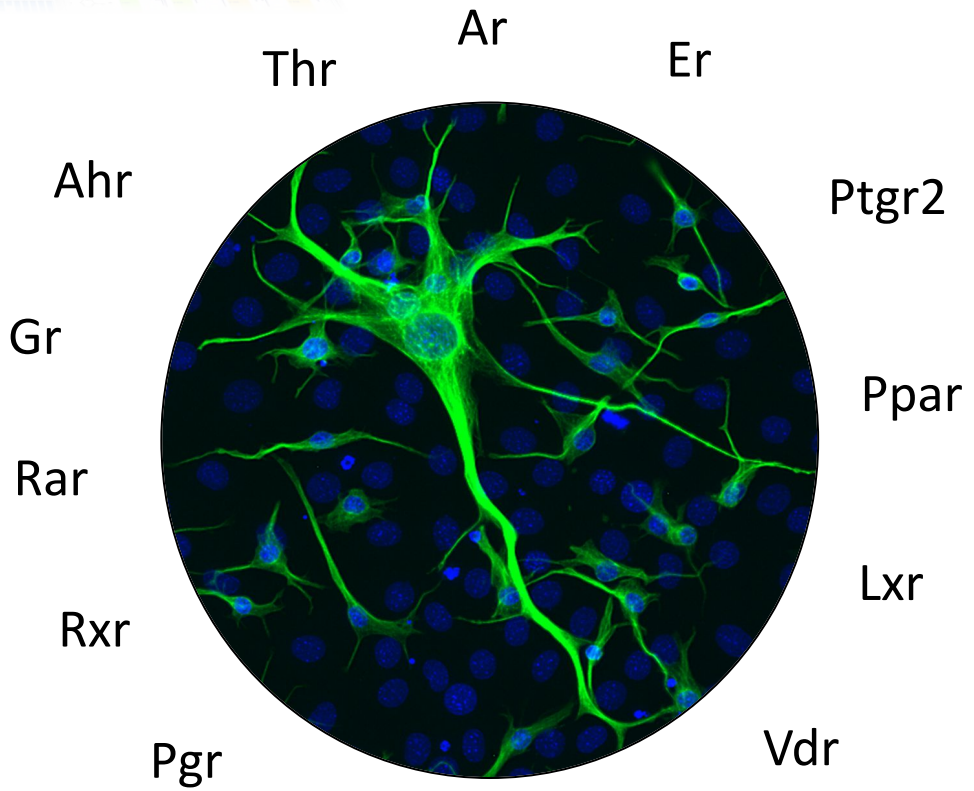
Omics integration (Metabolomics, Transcriptomics, Epigenomics)

Effects on cognition and behaviour

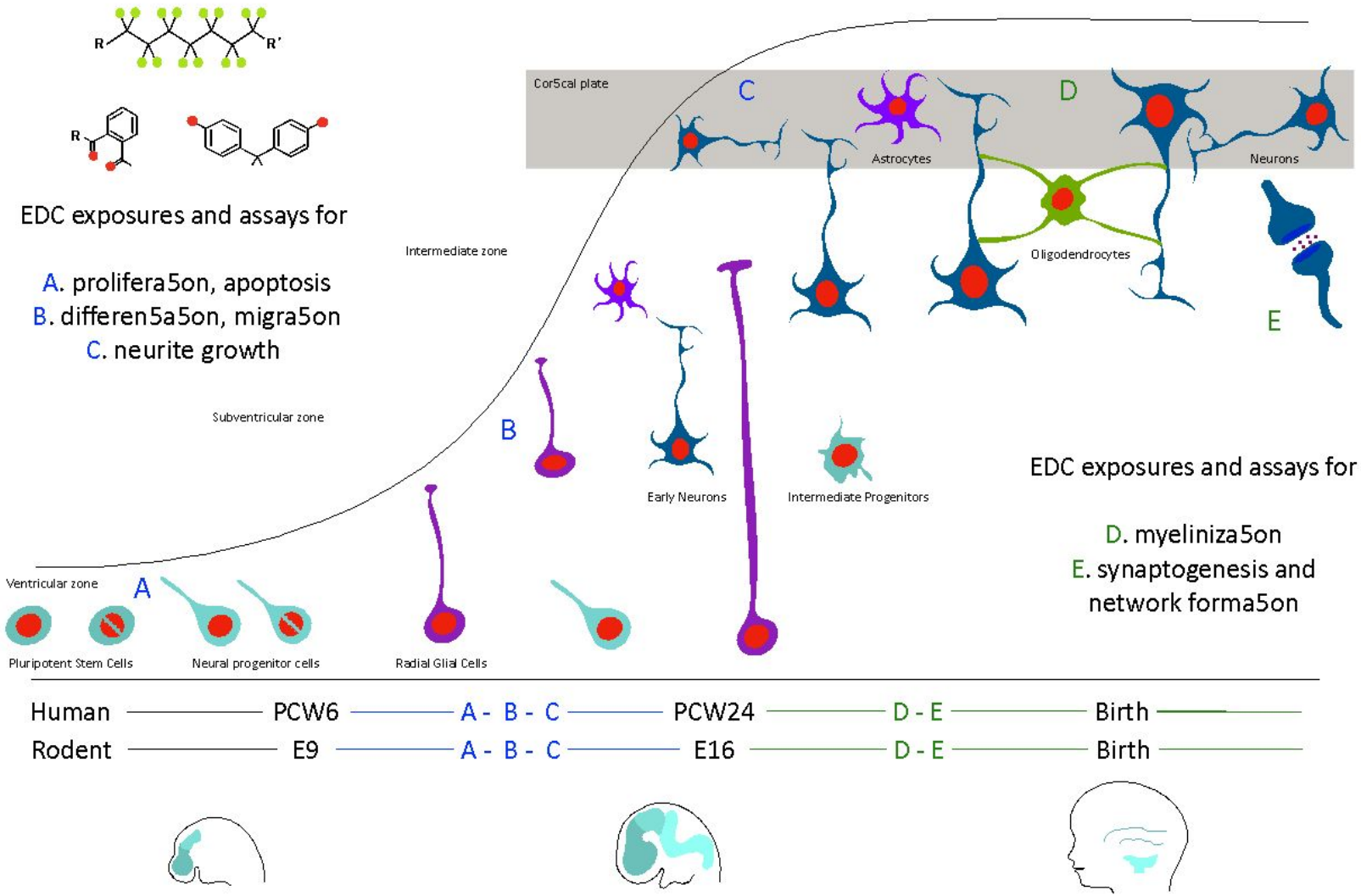
Selection of pathways



Chemical	Neurodevelopmental effects
DEP, DBP, BBzP, DEHP, DINP, DIDP, BPA, TCP, 3-PBA, PCB-180	Language delay
PFHxS, PFOS, PFOA, p,p'-DDE	Language delay, behaviour problems
Aldicarb, Permethrin, Methomyl	Behaviour problems
Endosulfan,	Behaviour and

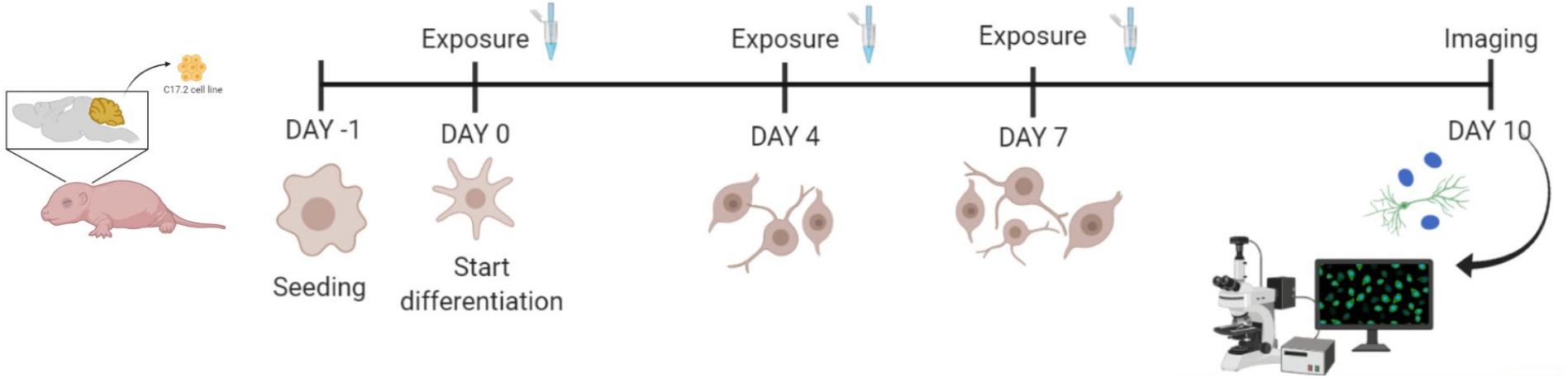


Are these targets involved in DNT key events?

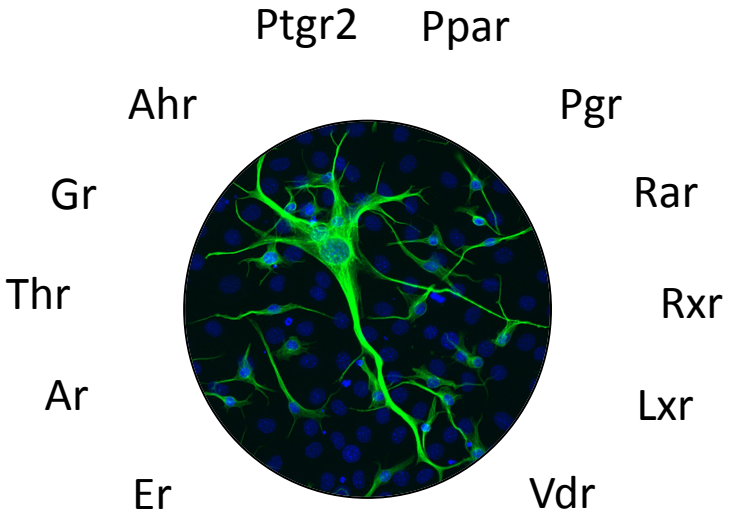


- Cell lines/primary cells
- Induced pluripotent stem cell-based models
- 3D models (spheres and brain organoids)
- rodent and human, female and male

The C17.2 cell line



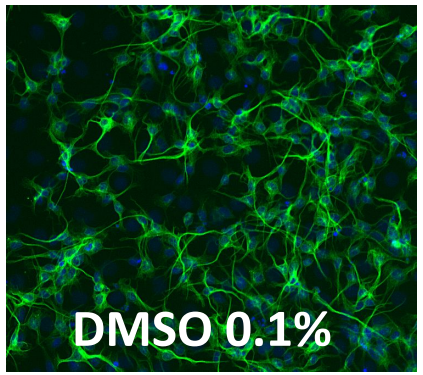
- Viability (AB)
- Neurite length
- Branching
- Percentage of neurons

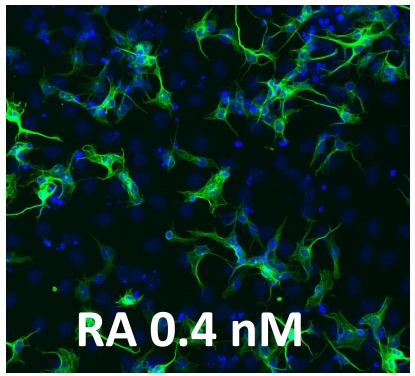


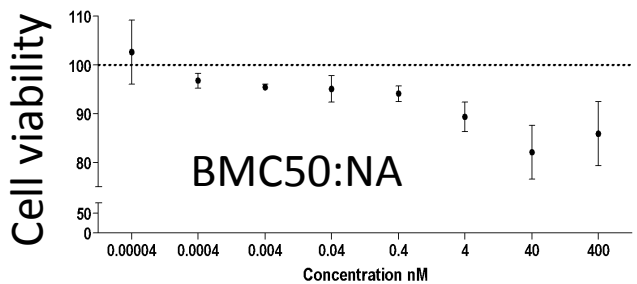
Exposure to (ant)agonist of hormonal pathways

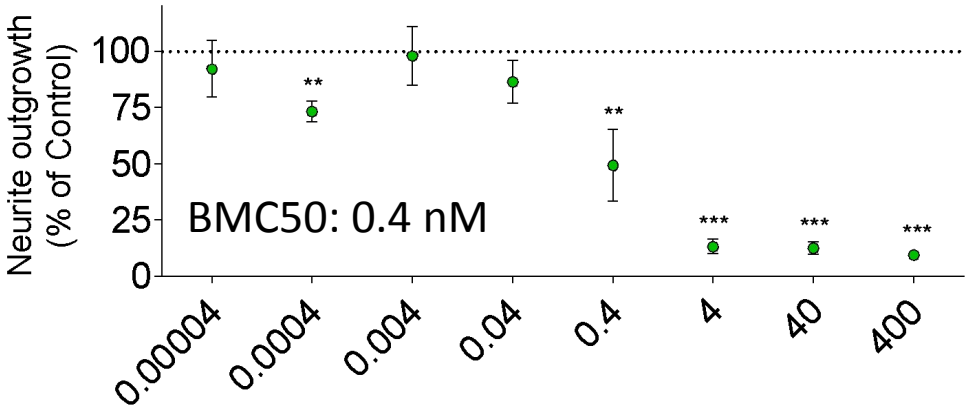
Andrea Cediel Ulloa

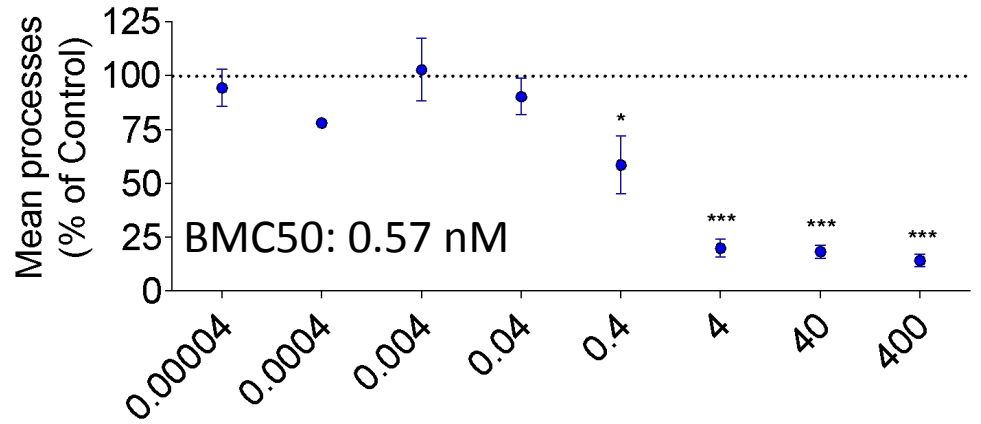
RAR-dependent neurite morphology in the C17.2 cell line

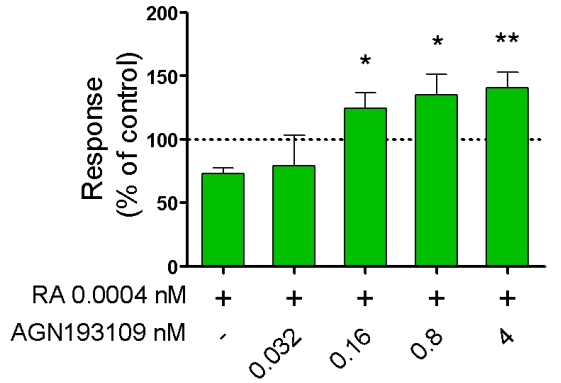


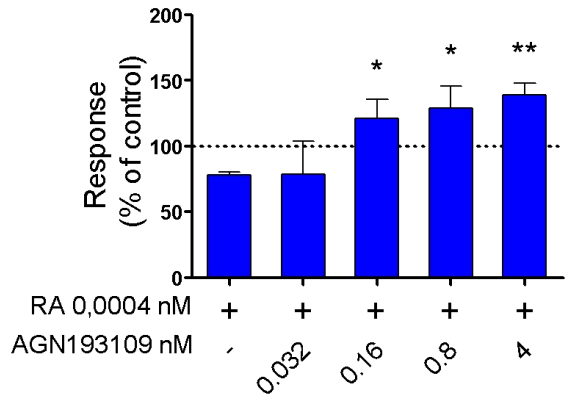






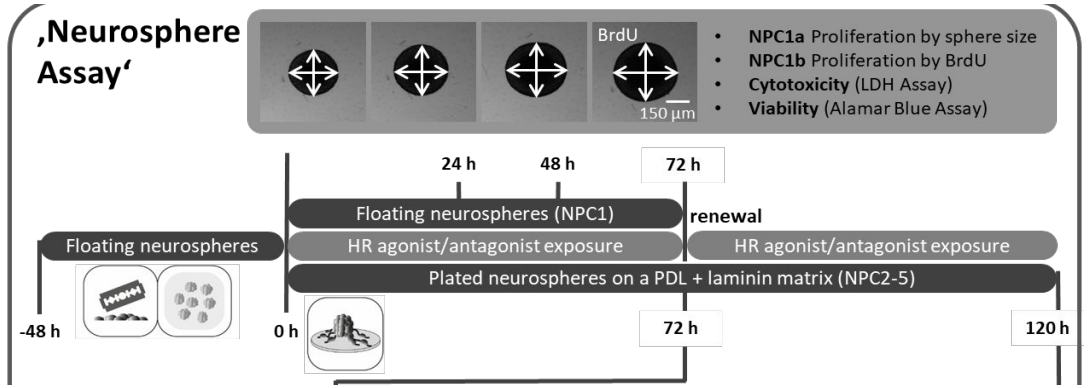


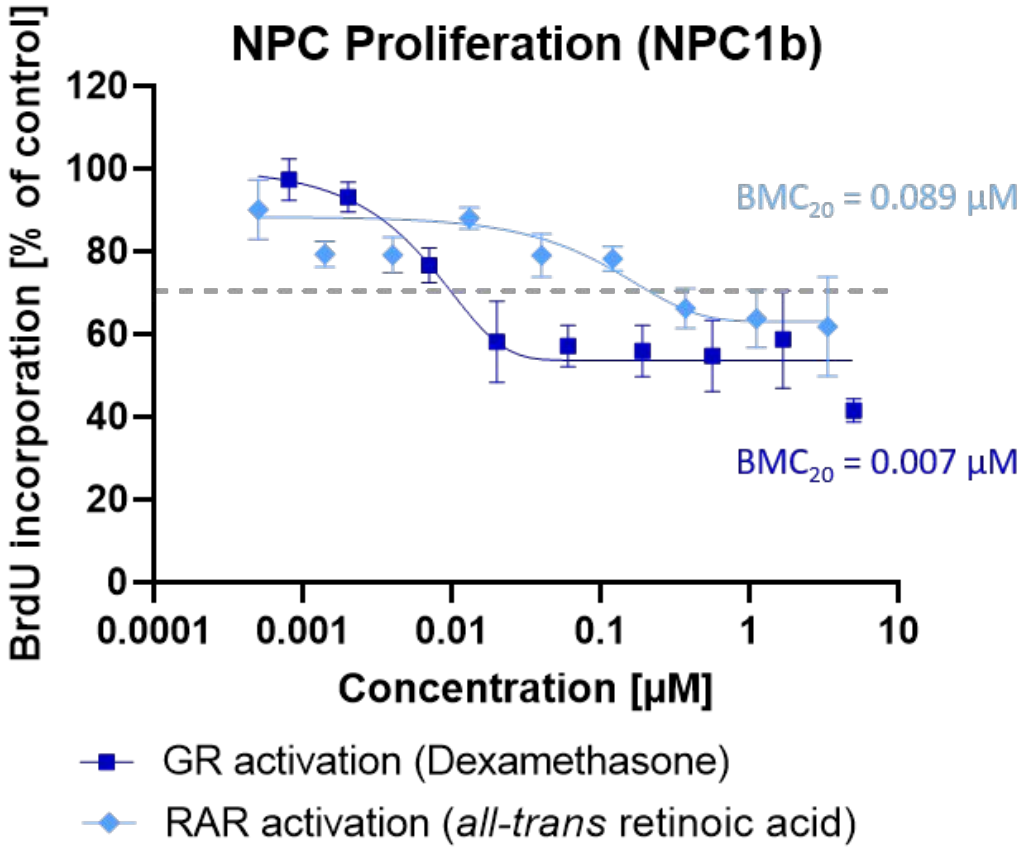




IUF
LEIBNIZ-INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG

The NPC1_RAR_GR assay





Katharina Koch, Kevin Schlüppmann, Ellen Fritsche

Many DNT KE are regulated by endocrine signalling

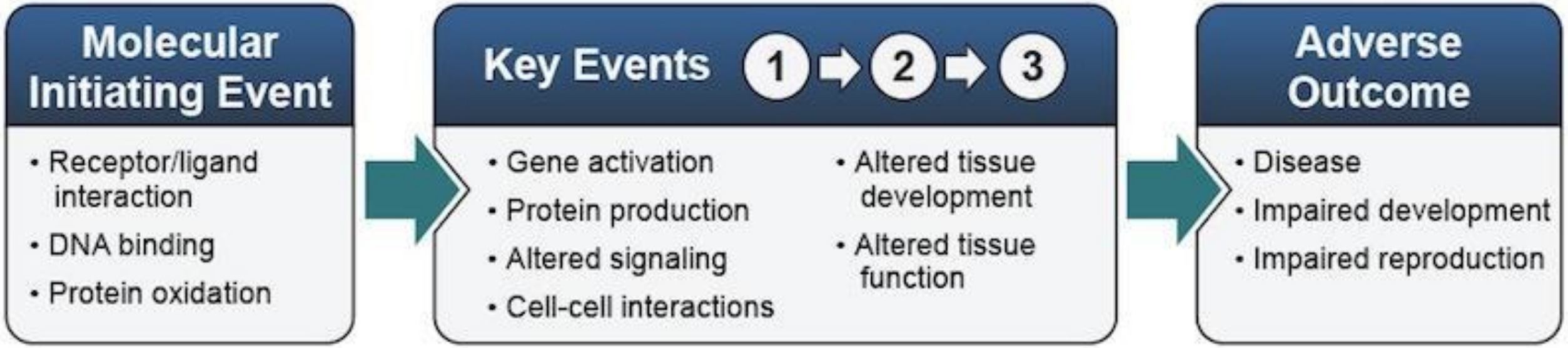
Key Events regulated by endocrine signaling		
	Decrease	*Increase*
NPC proliferation	**hGR, hRAR, mTR**	**mTR**
Neuronal differentiation	**mRXR, mRAR**	**hPPARγ, hLXR, hRXR, hGR**
Neurite outgrowth	**mRAR, mRXR, mRXR, mLXR, mPPARδ, mPPARδ hRXR, hRXR**	**mRAR, mRXR, hPPARβδ, hLXR, hTR**
Migration	**hRXR, hRXR, hTR**	
OPC differentiation	**hPPARα, hPPARβδ, hLXR, hPGE2R, hRAR, hVDR hGR, hPPARα, hTR, hER**	**mTR, hPPARγ**
Oligodendrocyte maturation (NPC6)		**TR (rat/human?)**

Agonist
Antagonist

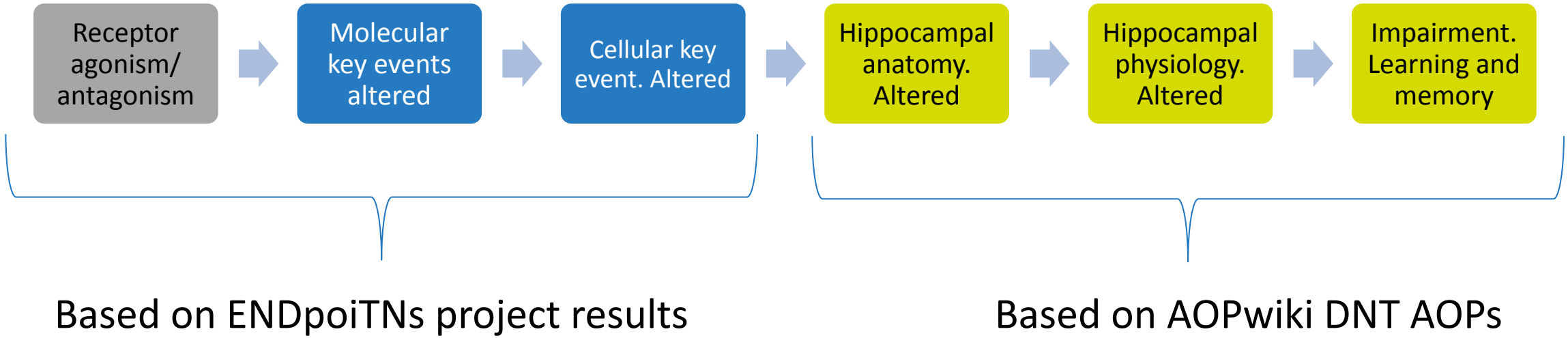
NPC: neural precursors
OPC: oligodendrocyte precursors

Adverse outcome pathways to integrate information

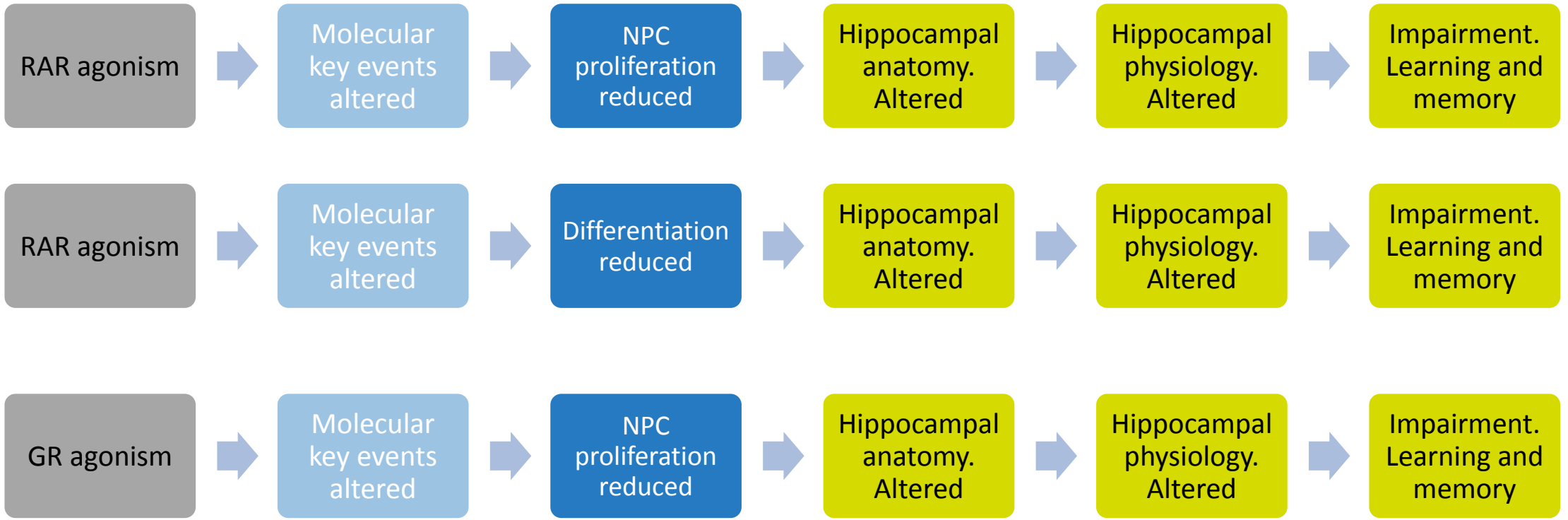
https://aopwiki.org/



AOPs in the context of ENDpoiNTs



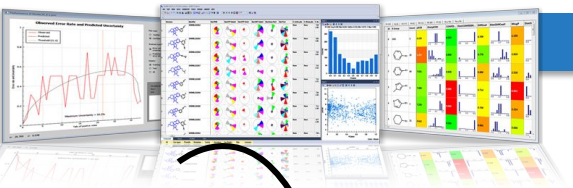
Cognition AOPs – RAR/GR pathways



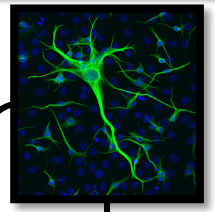
Diana Lupu

Receptor agonism/ antagonism → Molecular key events altered → Cellular key event. Altered → Hippocampal anatomy. Altered → Hippocampal physiology. Altered → Impairment. Learning and memory

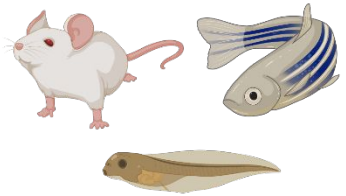
In silico



In vitro



In vivo



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Thank you!