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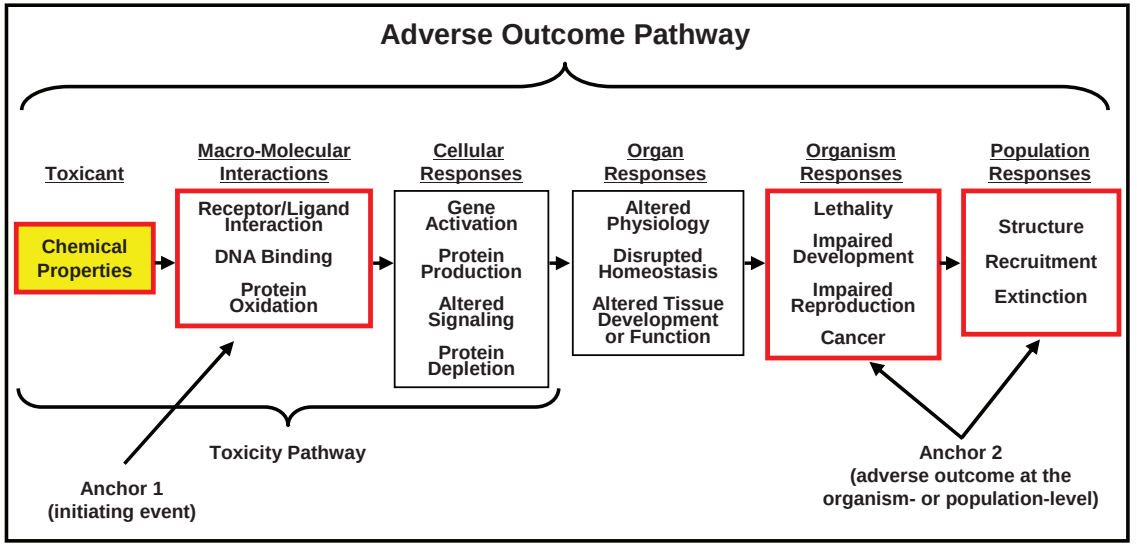
Human Neural Progenitor Cell (hNPC) Aging is mimicked by 3D Cultures in vitro: an 'Adverse Outcome Pathway' gains importance at susceptible life stages

Ellen Fritsche

Mitglied der Leibniz-Gemeinschaft

The Adverse Outcome Pathway (AOP) concept

- **The NRC report (2007)[1] supports a paradigm shift in toxicological risk assessment.**
- **AOPs provide a structure to organize existing knowledge on mode of action of compounds from the initiating event to the adverse outcome on the organ and organism level for supporting safety decisions[2].**



Ankley et al. 2010. Adverse Outcome Pathways: A conceptual framework to support ecotoxicology research and risk assessment. Env Tox Chem 29:730-41

[1]National Research Council. 2007. Toxicity testing in the 21st century: A vision and a strategy. National Academy of Sciences, Washington, DC.
[2]OECD Template and Guidance on developing and assessing the completeness of Adverse Outcome Pathways (2012) .

The AOP concept

EUROPEANCOMMISSION
JOINT RESEARCH CENTRE

SEURAT-1
Towards the Replacement of in vivo Repeated Dose Systemic Toxicity Testing

www.seurat-1.eu

Workshop on

"Using mechanistic information in developing the concept of the adverse outcome pathway (AOP) relevant to human neurotoxicity evaluation"

21 – 22 March 2013
JRC Ispra, Italy

Host: Maurice Wheelan, JRC
Chair: Anna Price, JRC

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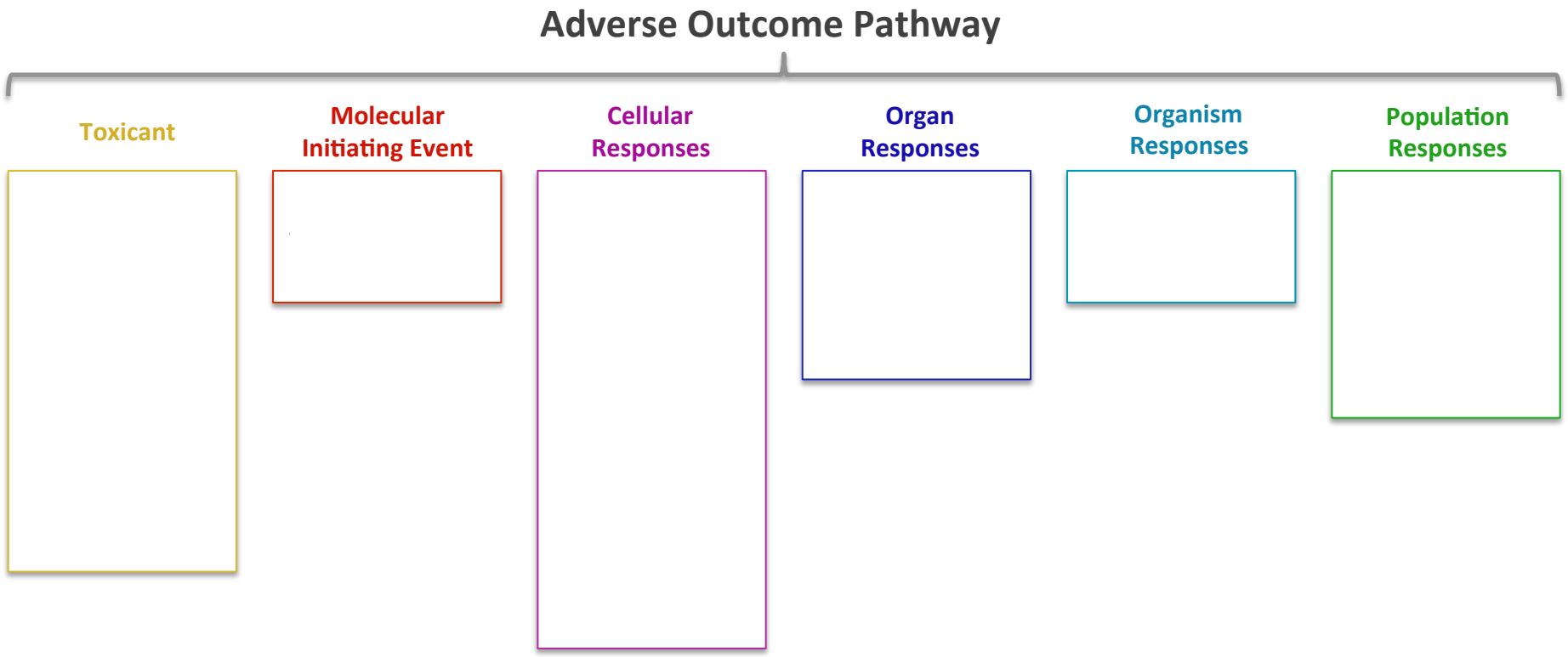
AOP on Impaired Adult Neurogenesis



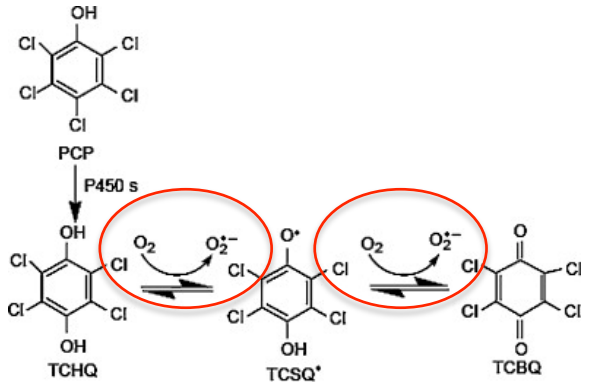
AOP on Impaired Adult Neurogenesis

Adverse Outcome Pathway

Toxicant

Reactive Oxygen Species (ROS; Redox Cyler, Pesticides, Metals, Ionizing Radiation, Chemothera-peutics)

$O_2 \cdot^-$ | $HO\cdot$ | $ROO\cdot$ | H_2O_2



AOP on Impaired Adult Neurogenesis

Adverse Outcome Pathway

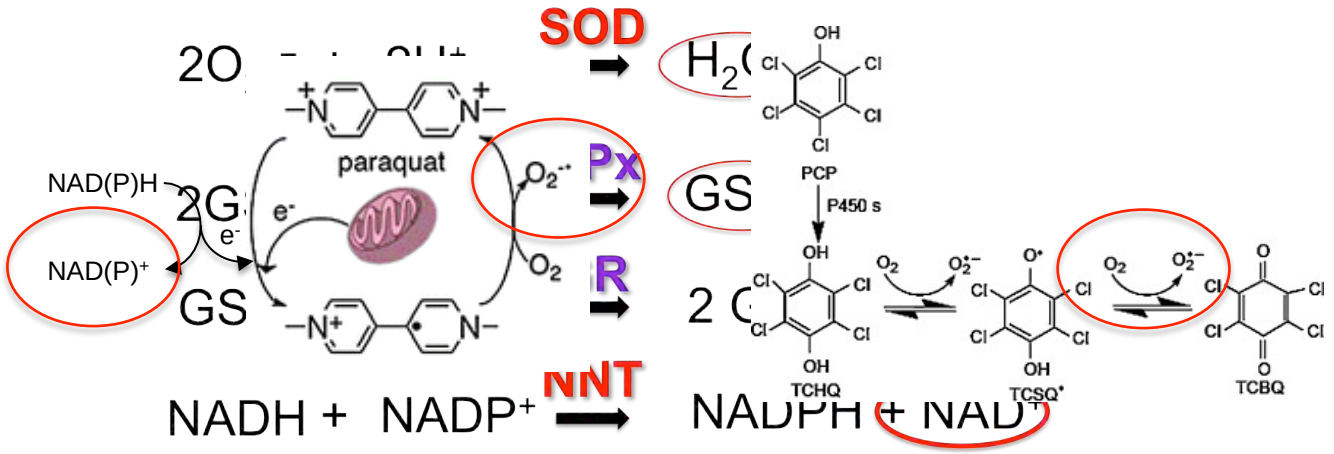
Toxicant

Reactive Oxygen Species (ROS; Redox Cyler, Pesticides, Metals, Ionizing Radiation, Chemothera-peutics)

Molecular Initiating Event

ROS causes formation of NAD^+

$O_2 \cdot^-$ | $HO\cdot$ | $ROO\cdot$ | H_2O_2

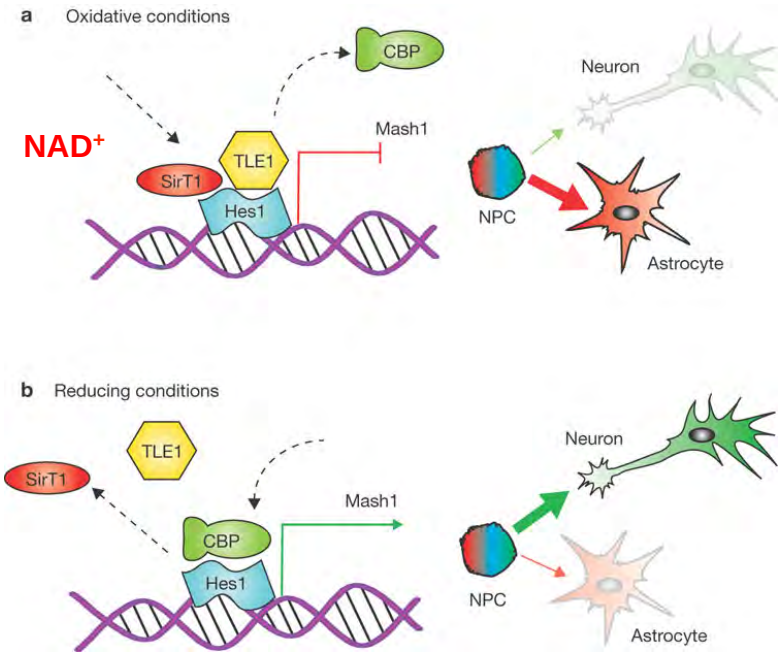


NNT=nicotinamide nucleotide transhydrogenase

AOP on Impaired Adult Neurogenesis

Adverse Outcome Pathway

Toxicant	Molecular Initiating Event	Cellular Responses
Reactive Oxygen Species (ROS; Redox Cyler, Pesticides, Metals, Ionizing Radiation, Chemothera-peutics)	ROS causes formation of NAD^+	· Activation of the HDAC Sirt1 · Co-repression of pro-neural genes



Libert et al. *Nature Cell Biology* 2008

AOP on Impaired Adult Neurogenesis

Adverse Outcome Pathway

Toxicant

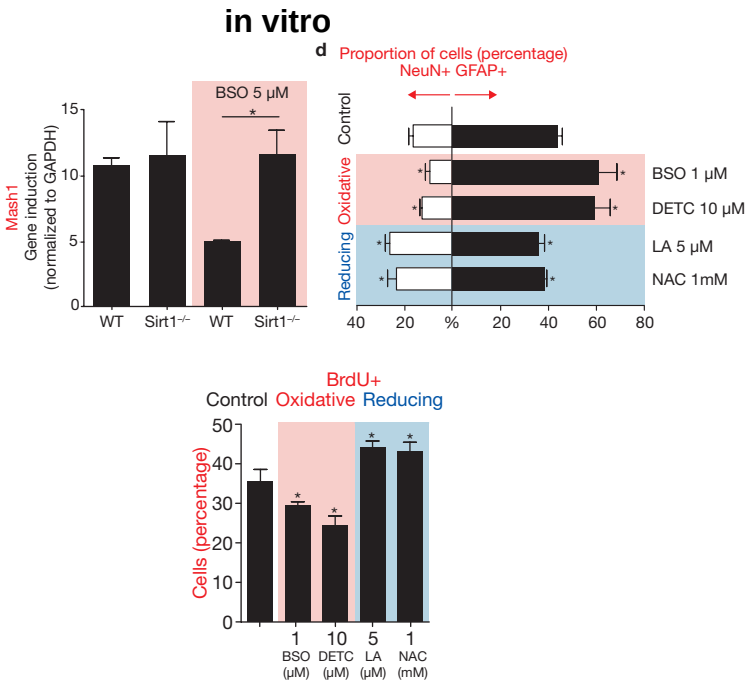
Reactive Oxygen Species (ROS; Redox Cyler, Pesticides, Metals, Ionizing Radiation, Chemothera-peutics)

Molecular Initiating Event

ROS causes formation of NAD^+

Cellular Responses

- Activation of the HDAC Sirt1
- Co-repression of pro-neural genes
- Decrease in NPC function in the hippocampus: Proliferation and Neuronal Differentiation



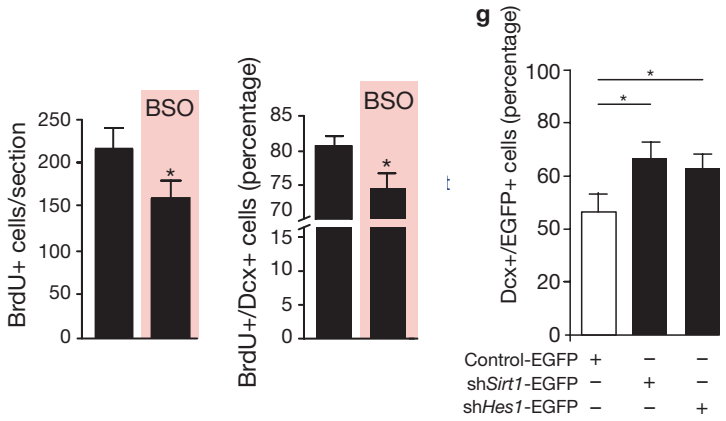
Prozorovski et al. *Nature Cell Biology* 2008

AOP on Impaired Adult Neurogenesis

Adverse Outcome Pathway

Toxicant	Molecular Initiating Event	Cellular Responses	Organ Responses
Reactive Oxygen Species (ROS; Redox Cyler, Pesticides, Metals, Ionizing Radiation, Chemothera-peutics)	ROS causes formation of NAD^+	· Activation of the HDAC Sirt1 · Co-repression of pro-neural genes · Decrease in NPC function in the hippocampus: Proliferation and Neuronal Differentiation	Decreased NPC function in the hippocampus

in vivo



Prozorovski et al. *Nature Cell Biology* 2008

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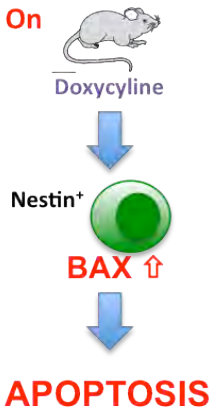
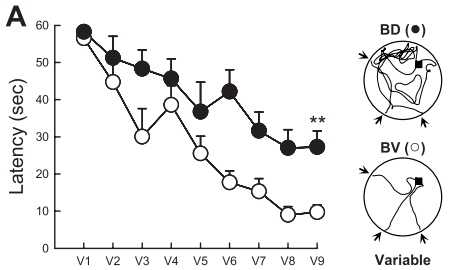
AOP on Impaired Adult Neurogenesis

Adverse Outcome Pathway

Toxicant	Molecular Initiating Event	Cellular Responses	Organ Responses	Organism Responses
Reactive Oxygen Species (ROS; Redox Cyler, Pesticides, Metals, Ionizing Radiation, Chemothera-peutics)	ROS causes formation of NAD^+	· Activation of the HDAC Sirt1 · Co-repression of pro-neural genes · Decrease in NPC function in the hippocampus: Proliferation and Neuronal Differentiation	Decreased NPC function in the hippocampus	Learning and memory impairment





Dupret et al. PLOS One 2008

AOP on Impaired Adult Neurogenesis

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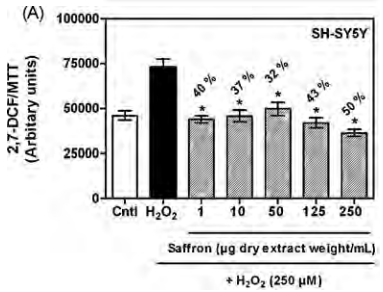
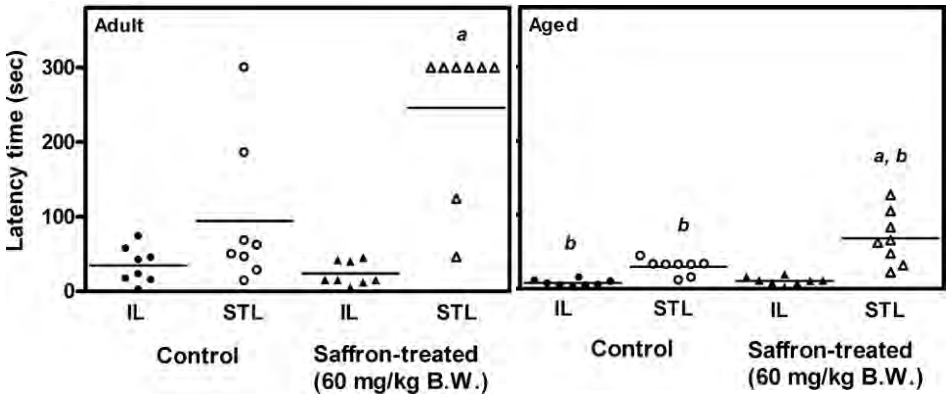


Passive Avoidance Task – Step Through Latency (STL) as a measure for Long-Term Memory



Papandreou et al. Beh Brain Res 2008

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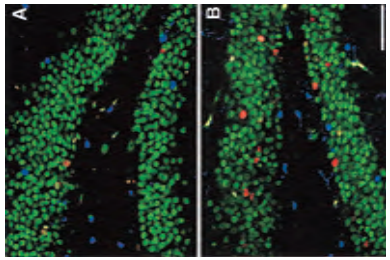
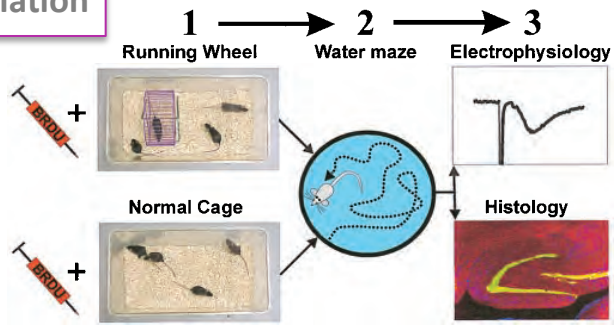
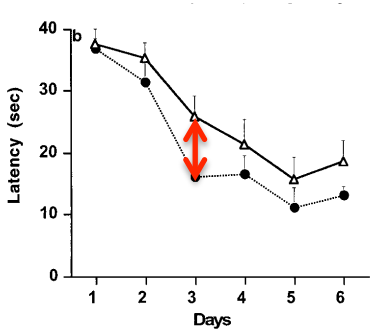


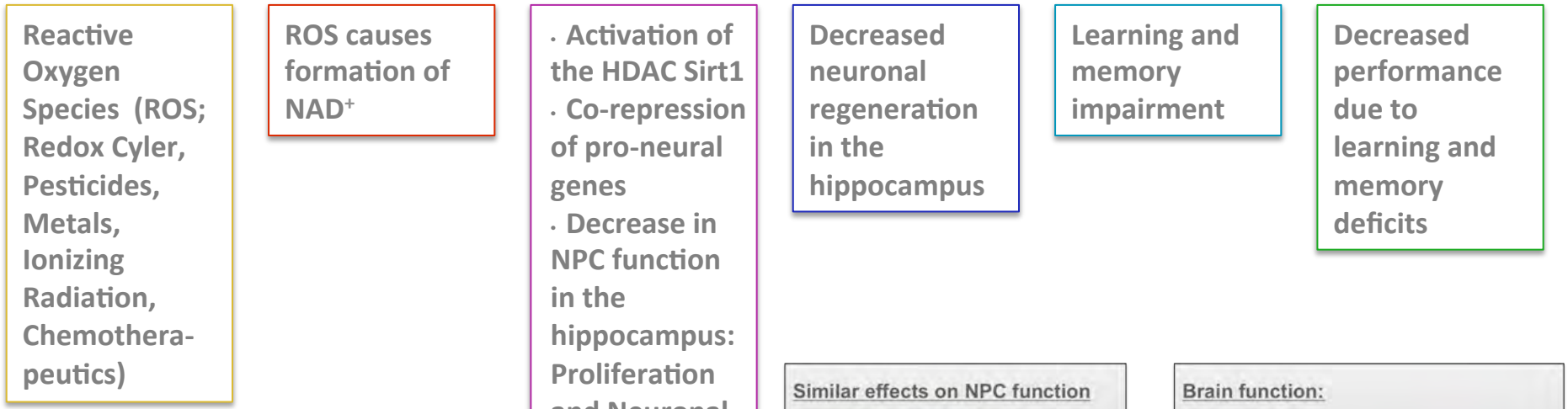




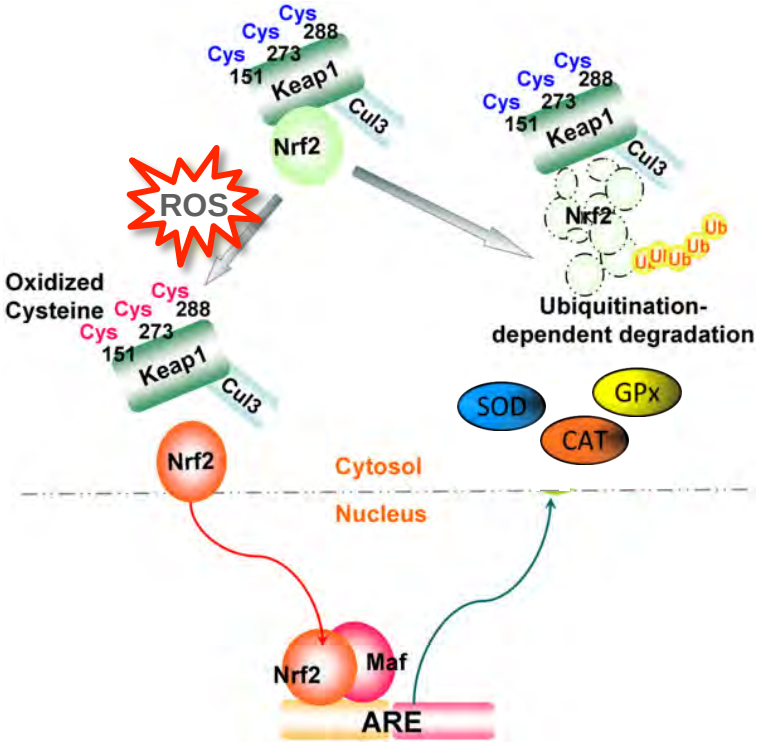
van Praag et al. PNAS 2006

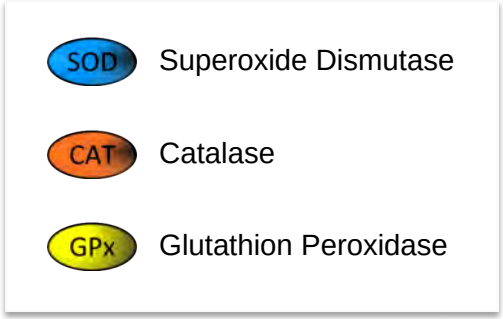
AOP on Impaired Adult Neurogenesis

Adverse Outcome Pathway



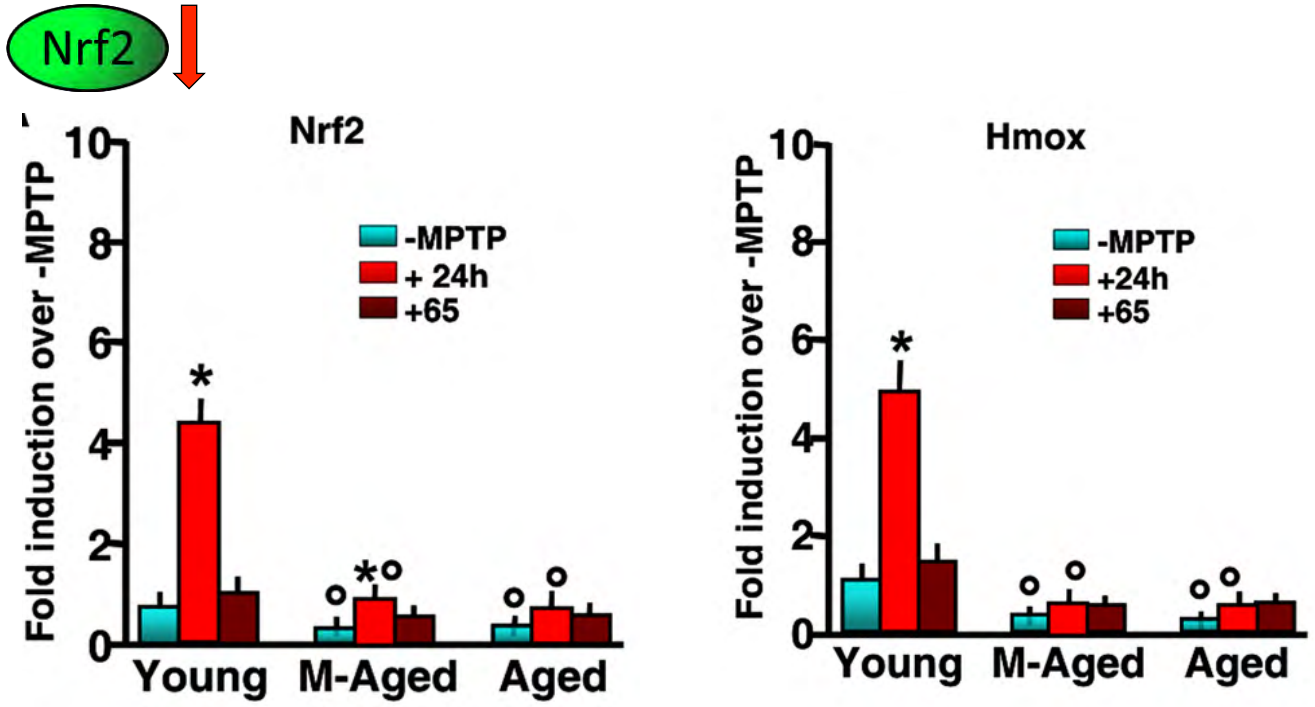
The Nrf2 Signaling Pathway





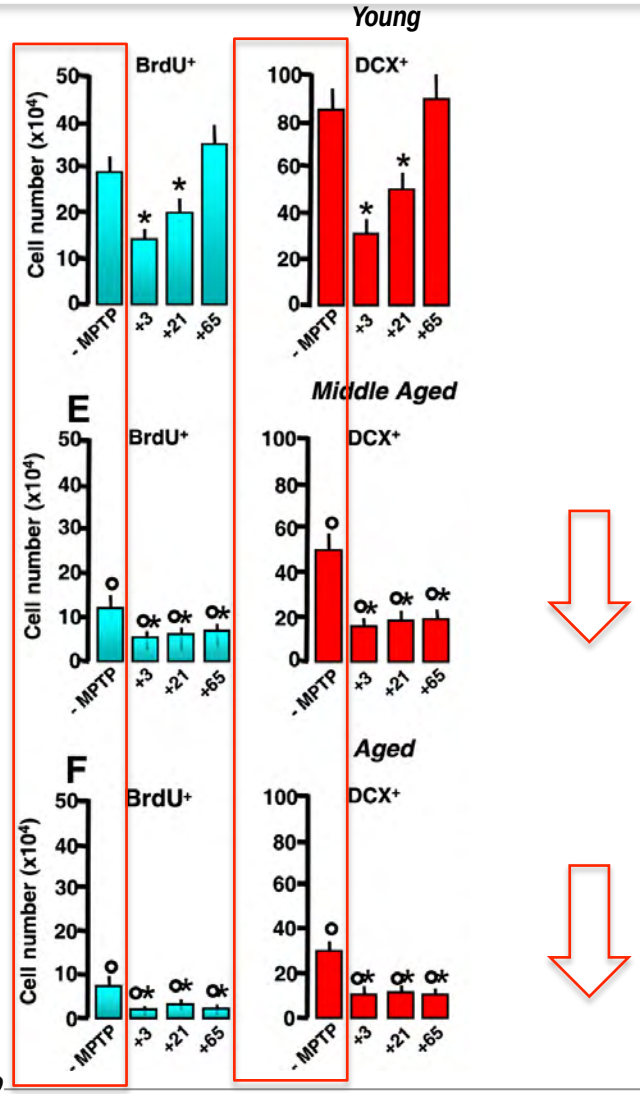
mod. after Ray et al. Cell. Signal. 2012

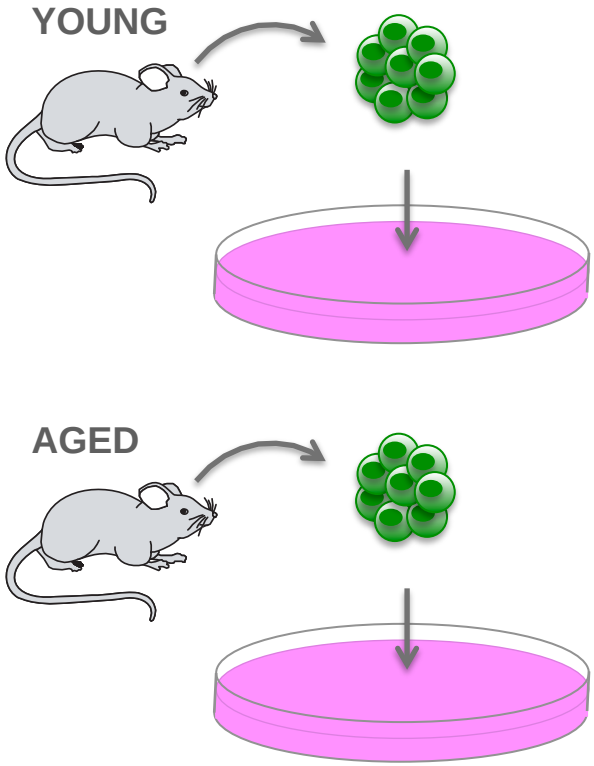
Nrf2 Expression & Nrf2-dpdt gene expression decreases in aged SVZ in mice in vivo



L'Episcopo et al. J Neurosci 2013

Age-related NPC function can be carried from brains *in vivo* to the dish *in vitro*





- NPC proliferation ↓
- DCX⁺ & Tuji1⁺ cells ↓

L'Episcopo et al. J Neurosci 2013

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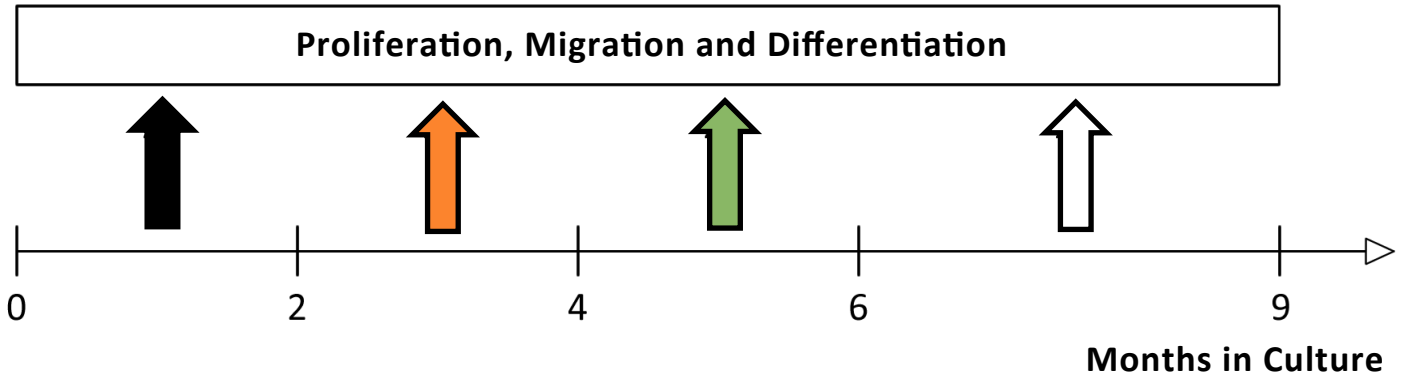
Questions

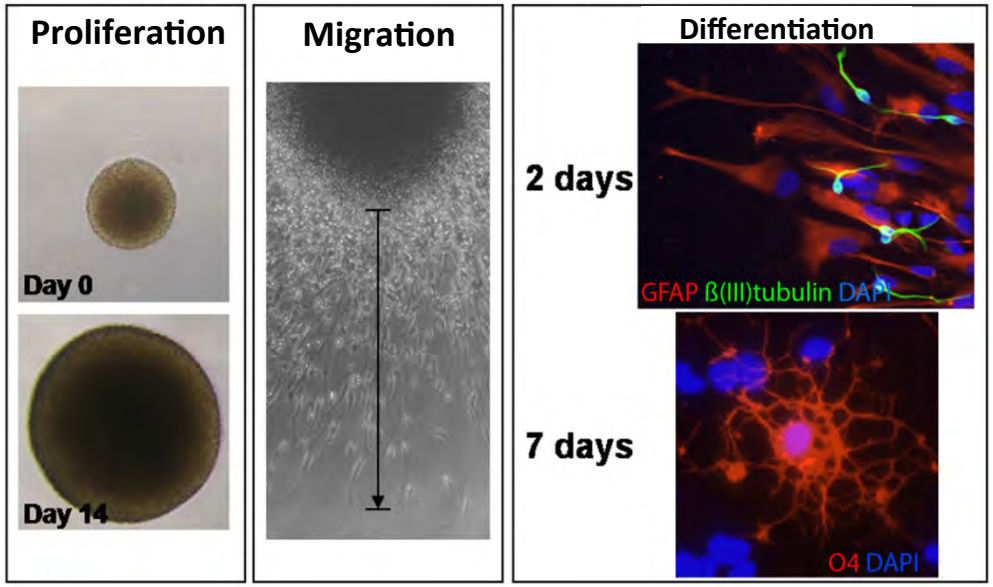
1. Is it possible to establish a human NPC aging assay *in vitro*?

2. What are the molecular mechanisms driving human NPC aging? Are they reflected by the AOP on 'Impaired Adult Neurogenesis'?

3. Can we develop a medium throughput test based on human NPC for assessing chemical effects on human adult neurogenesis?

The Human Neurosphere Aging Assay

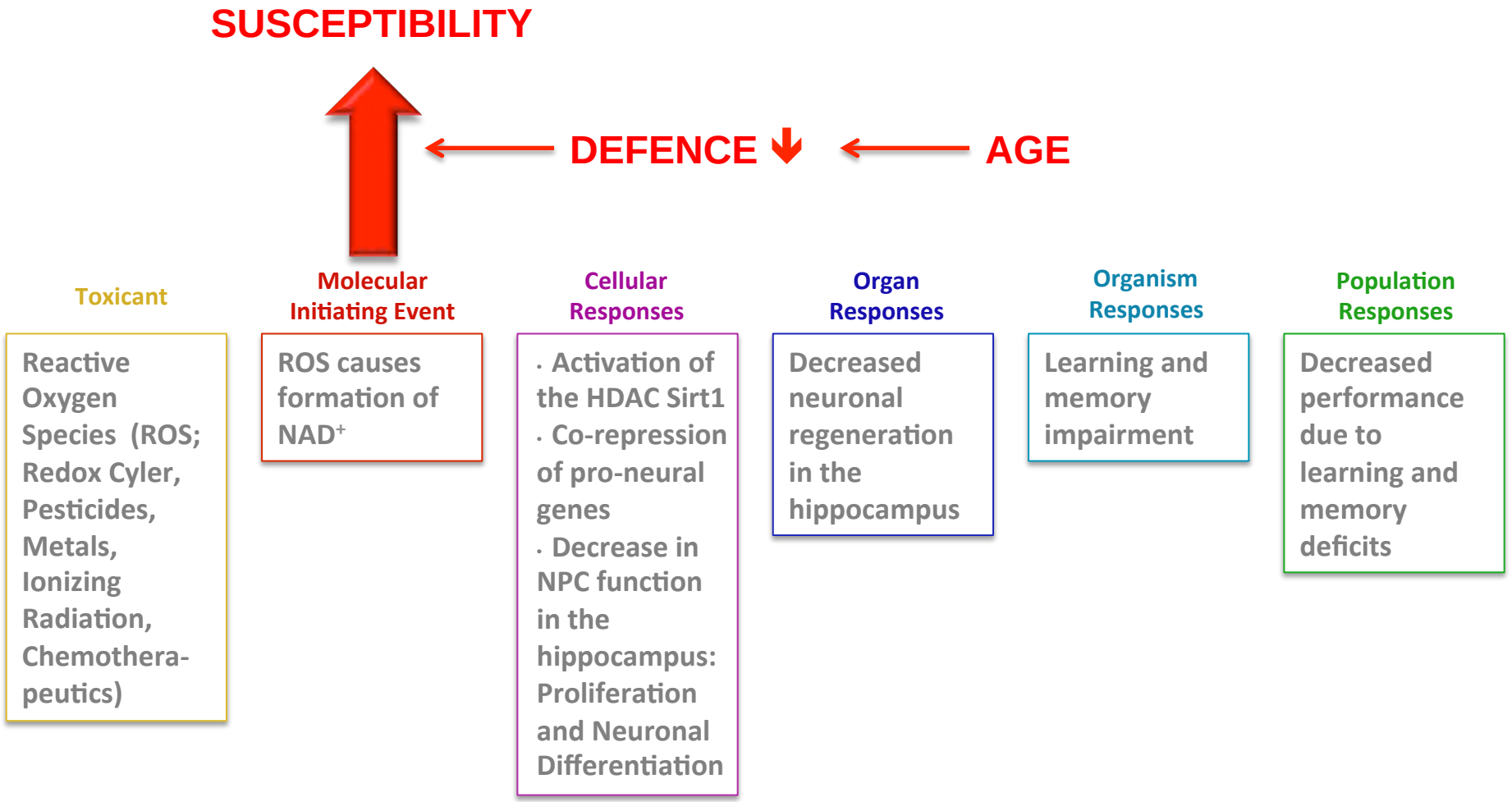




Fritsche et al. Environ Health Perspect 2005
Moors et al. Toxicol Appl Pharmacol 2007
Moors et al. Environ Health Perspect 2009
Moors et al. Genes & Immunity 2010
Tegenge et al. Cell. Mol. Life Sci. 2010
Schreiber et al. Environ Health Perspect 2010
Gassmann et al. Environ Health Perspect 2010
Verner et al. Toxicol in Vitro 2011
Fritsche et al. Methods Mol Biol 2011
Gassmann et al. Toxicol in Vitro 2012

Breier,...Fritsche et al. Neurotoxicol Teratol 2009

Age modulates AOP on Impaired Adult Neurogenesis



Questions

1. Is it possible to establish a human NPC aging assay *in vitro*?

2. What are the molecular mechanisms driving human NPC aging? Are they reflected by the AOP on 'Impaired Adult Neurogenesis'?

3. **Can we develop a medium throughput test based on human NPC for assessing chemical effects on human adult neurogenesis?**

Applications of the Neurosphere Assay

- Medium Throughput:

Toxicology in Vitro 26 (2012) 993–1000

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

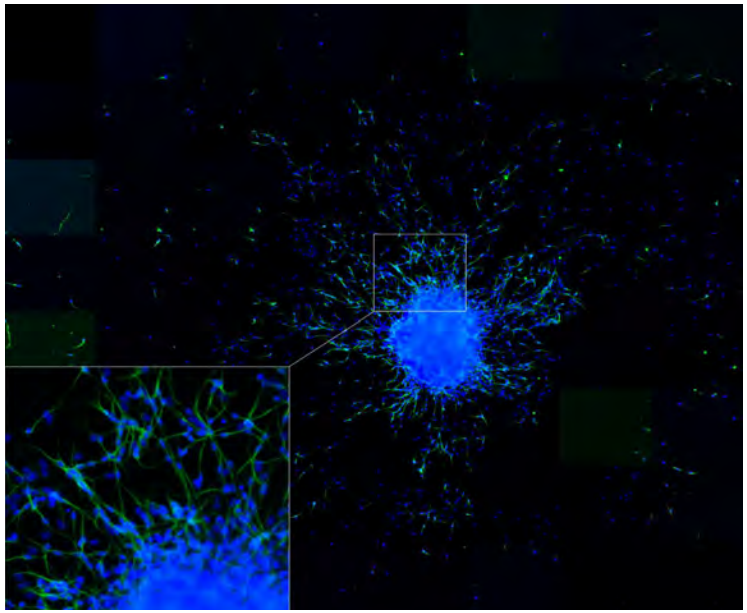
journal homepage: www.elsevier.com/locate/toxinvit

Automated neurosphere sorting and plating by the COPAS large particle sorter is a suitable method for high-throughput 3D *in vitro* applications

K. Gassmann [a,1], J. Baumann [a,1], S. Giersiefer [a,1], J. Schuwald [a,1], T. Schreiber [a], H.F. Merk [b], E. Fritsche [a,b,*]

[a] *Leibniz Research Institute for Environmental Medicine, Department Molecular Toxicology, Auf'm Hennekamp 50, 40225 Duesseldorf, Germany*
[b] *Universitaetshautklinik, RWTH Aachen, Aachen, Germany*

- High Content Image Analyses:



- Cell Biological or Molecular Endpoints reflecting AOP on 'Impaired Adult Neurogenesis'
- Chemical Testing with Species Comparison: **Poster**

Acknowledgements

Dr. Marta Barenys
Dr. Kathrin Gassmann
Dr. Julia Tigges
Dr. Susanne Giersiefer
Dr. Janette Schuwald
Katharina Dach
Martin Schmuck
Maxi Hofrichter
Katerina Kefalaki
Stefan Masjosthusmann
Thomas Temme
Denise de Boer
Ulrike Hübenthal

Prof. Dr. Orhan Aktas, HHU Düsseldorf
Prof. Dr. Brigitte Royer-Pokora HHU Düsseldorf
Prof. Kai Stühler, HHU Düsseldorf

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Thank you for your attention!