

**AN INTERSPECIES COMPARISON OF
TOXICITY PATHWAYS MEDIATING
NEURODEVELOPMENTAL TOXICITY IN
NEUROSPHERES - AND NOVEL
COMPUTATIONAL APPROACHES FOR HIGH
CONTENT IMAGE ANALYSES (HCA)**

Ellen Fritsche

EPA's Computational Toxicology Communities of Practice

September 25th 2014

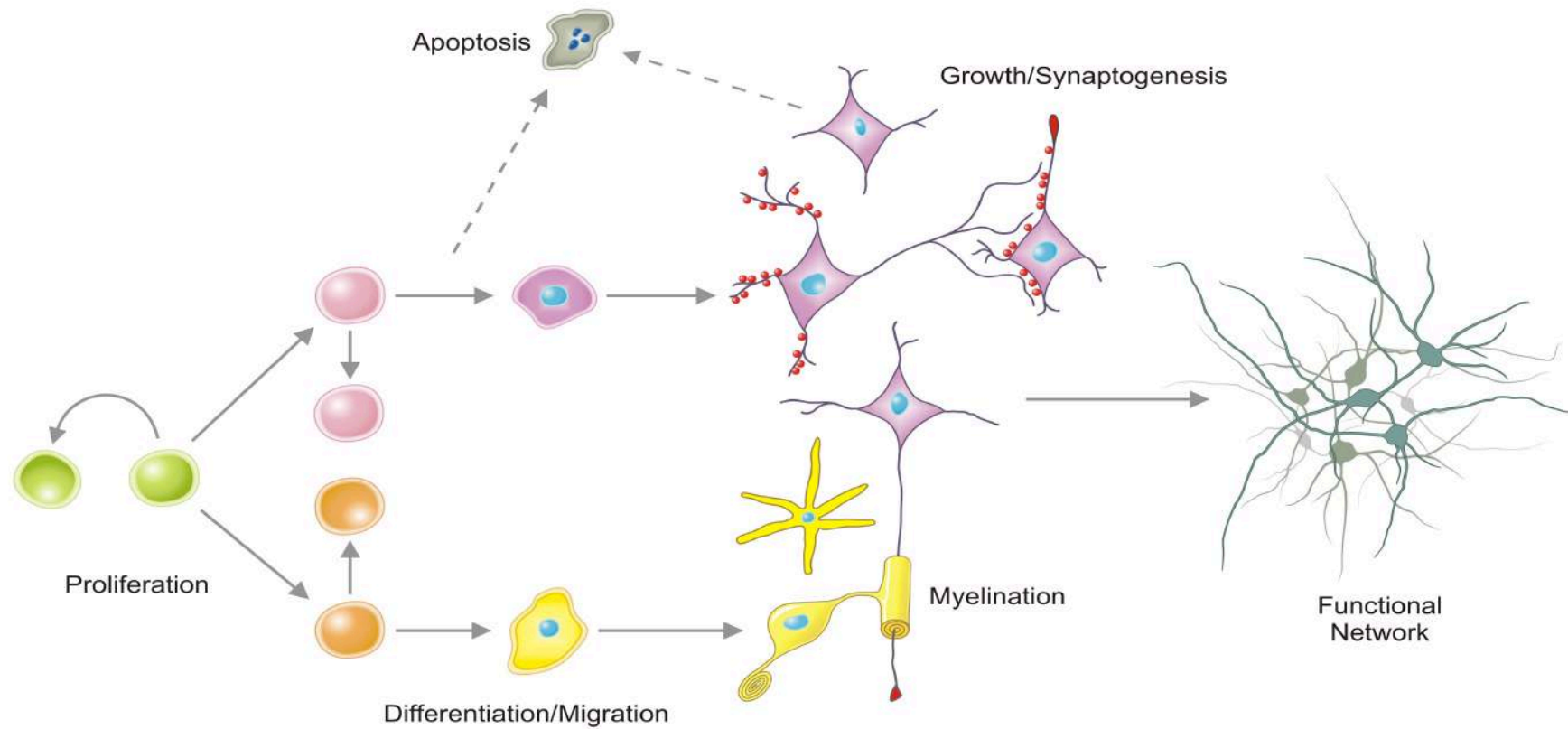
IUF

LEIBNIZ-INSTITUT
FÜR UMWELT-
MEDIZINISCHE
FORSCHUNG

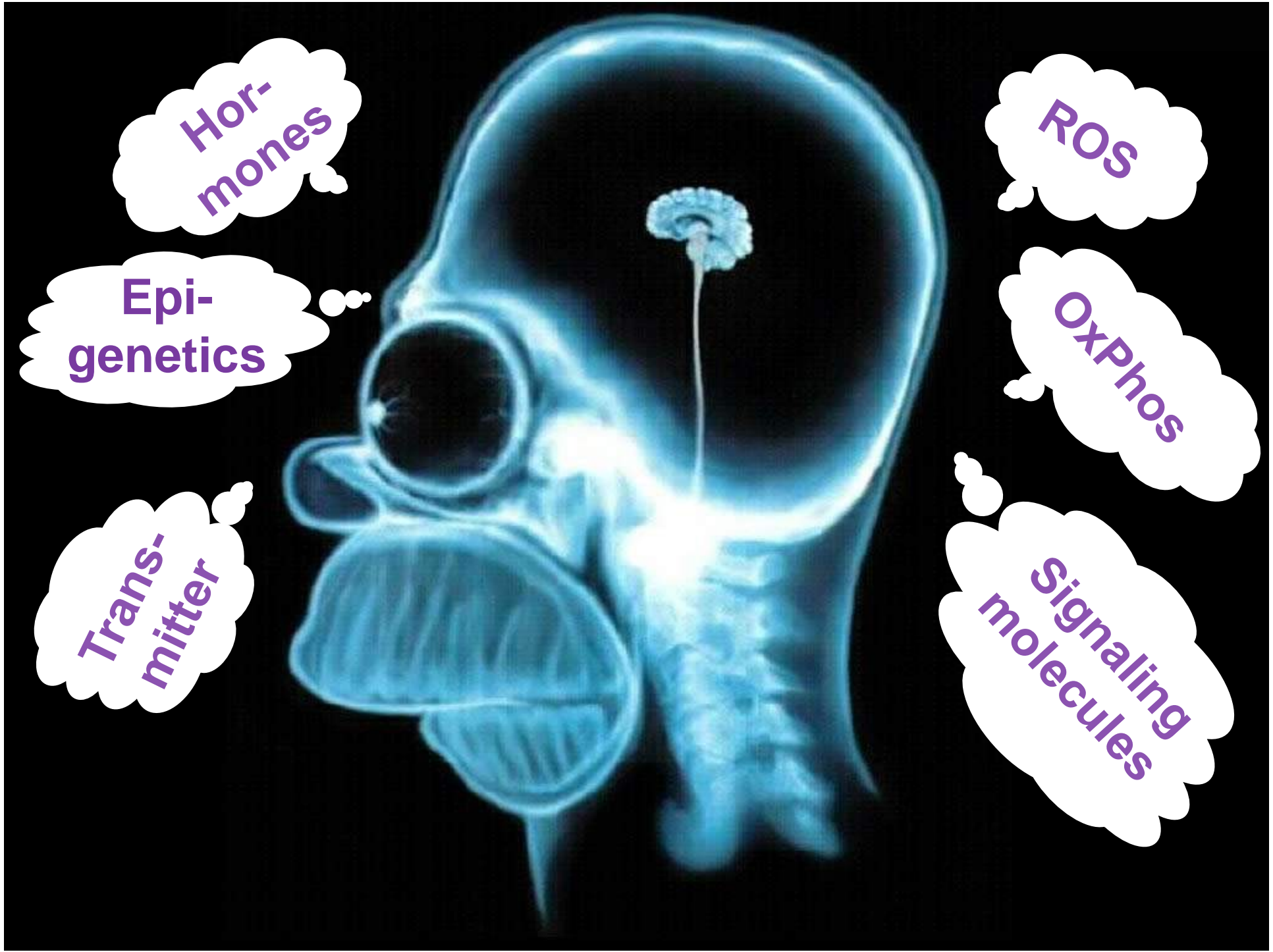
Mitglied der

Leibniz
Leibniz-Gemeinschaft

Cell Biological Processes performed by NPC



With courtesy from **William Mundy, U.S. Environmental Protection Agency** and **John Havel, SRA International, Inc.**



Hor-
mones

Epi-
genetics

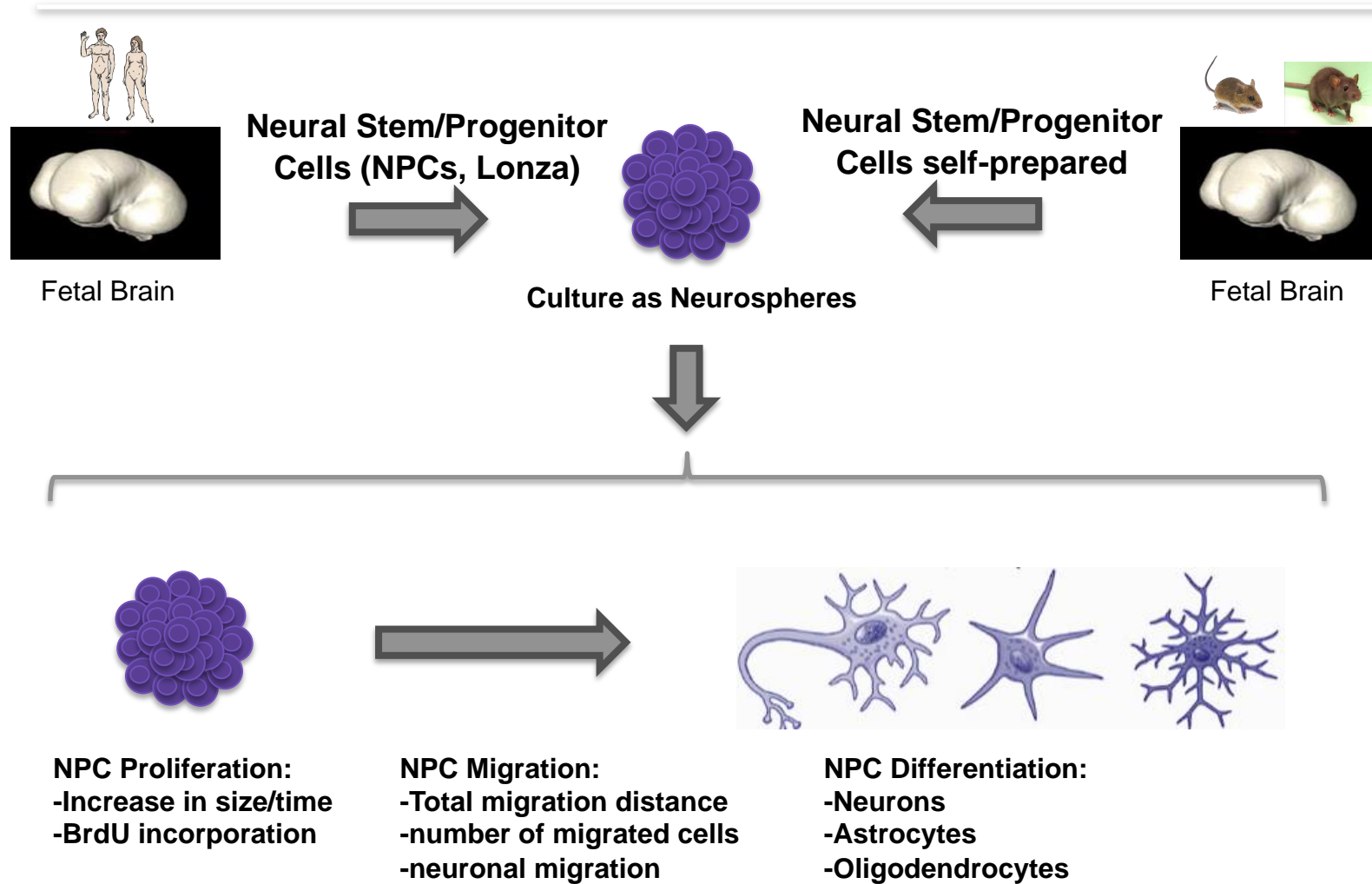
Trans-
mitter

ROS

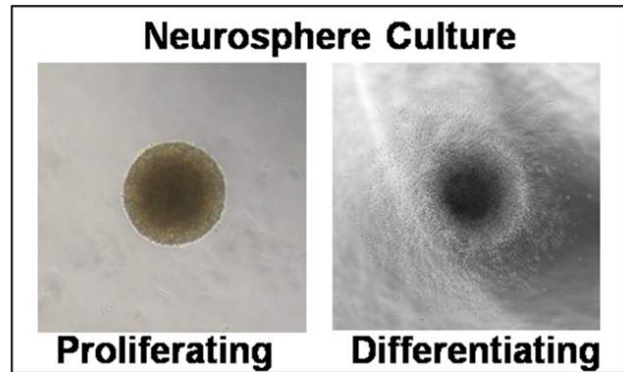
OxPhos

Signaling
molecules

The Neurosphere Method



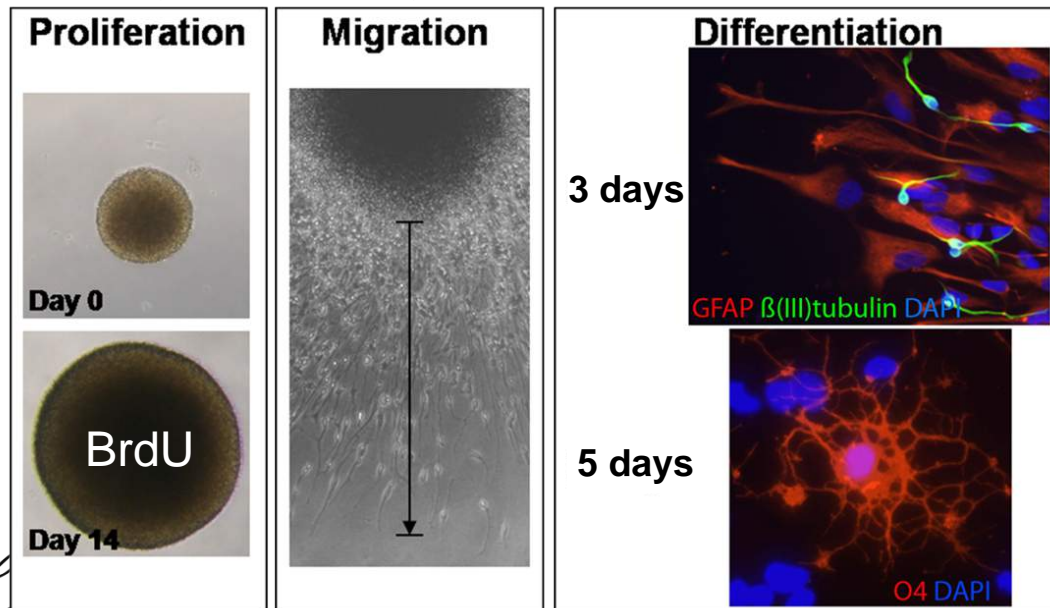
The 'Neurosphere-Assay'



Determination of Viability



Apoptosis

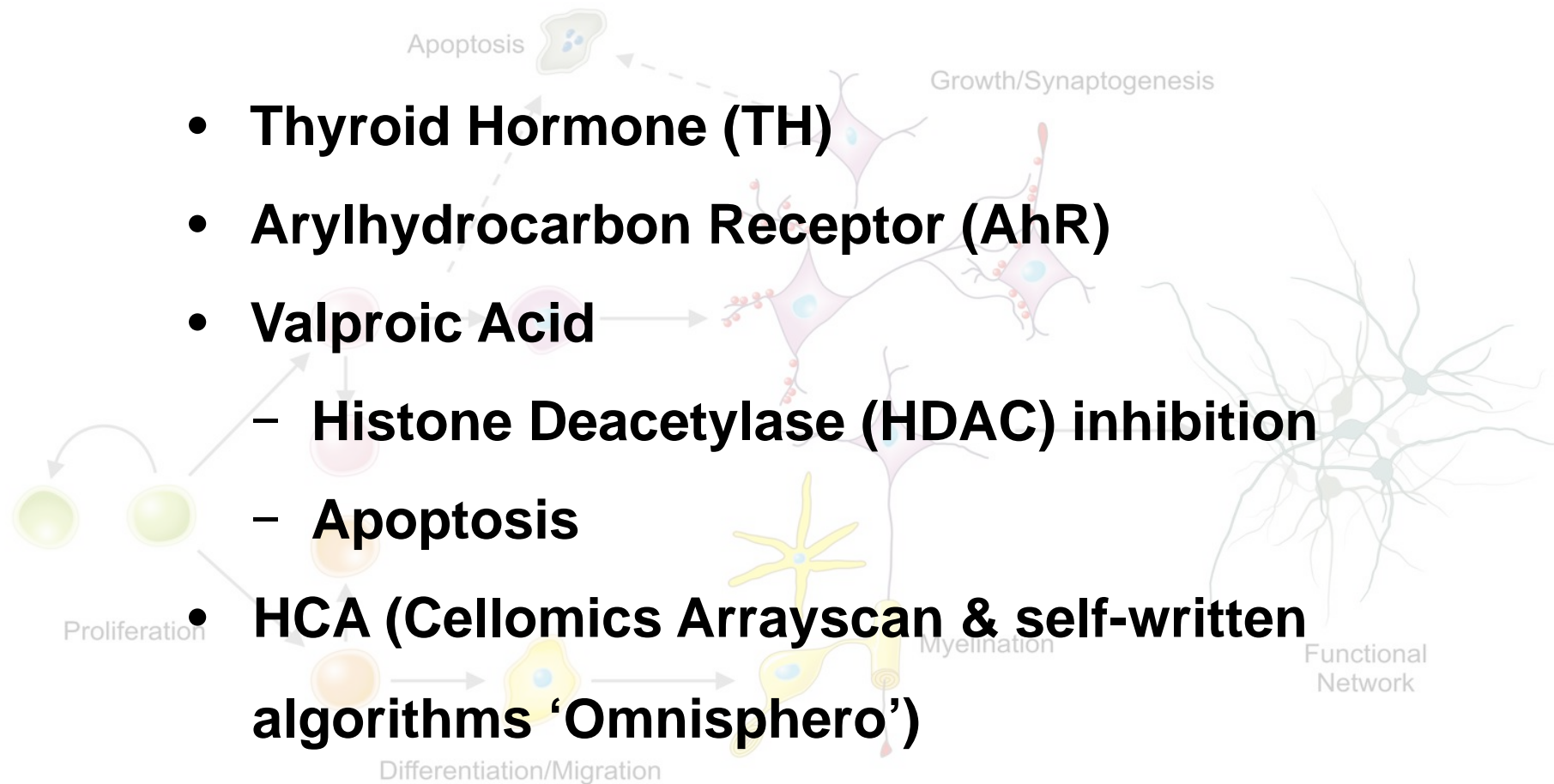


Fritsche et al. Environ Health Perspect 2005
 Moors et al. Toxicol Appl Pharmacol 2007
 Moors et al. Environ Health Perspect 2009
 Moors et al. Genes & Immunity 2010
 Tegenge et al. Cell. Mol. Life Sci. 2010
 Schreiber et al. Environ Health Perspect 2010
 Gassmann et al. Environ Health Perspect 2010
 Verner et al. Toxicol in Vitro 2011
 Fritsche et al. Methods Mol Biol 2011
 Gassmann et al. Toxicol in Vitro 2012
 Bal-Price et al. ALTEX 2012
 Baumann et al. Curr. Protoc. Toxicol. 2014
 Gassmann et al. Arch. Toxicol. 2014
 Fritsche Methods Pharmacol. Toxicol. 2014
 Alépée et al. ALTEX 2014

From Breier...Fritsche.. et al. *Neurotoxicol Teratol* 2009

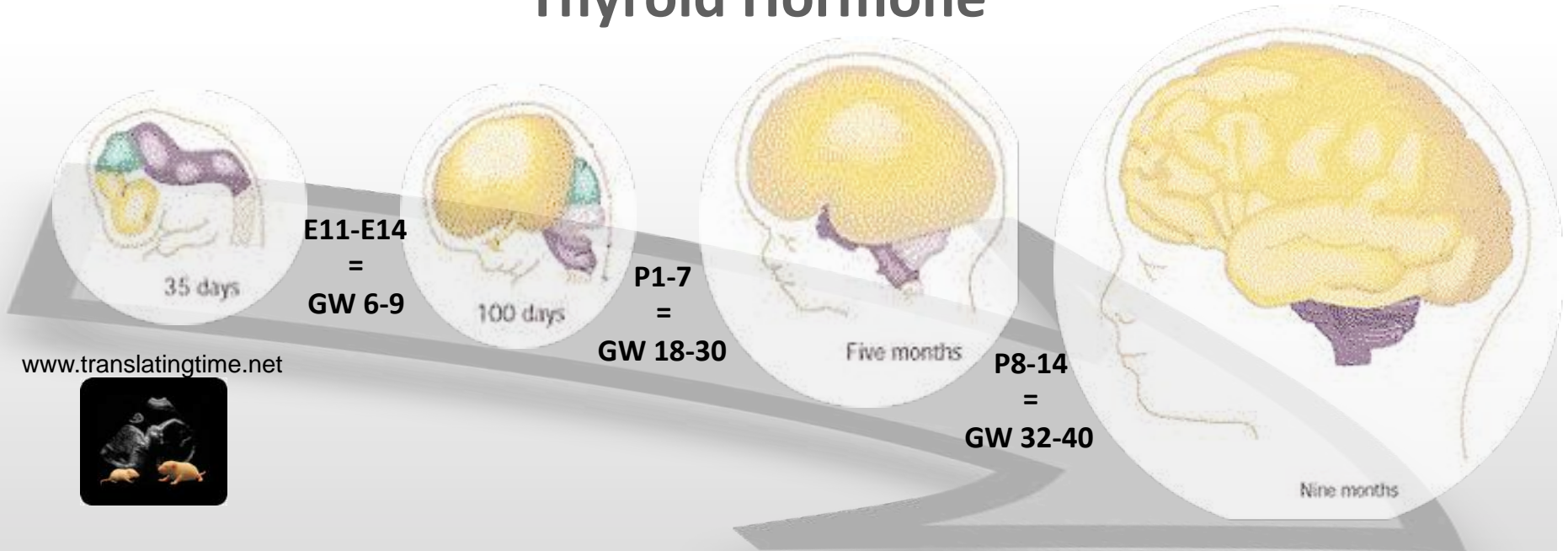
Erasmus

Outline

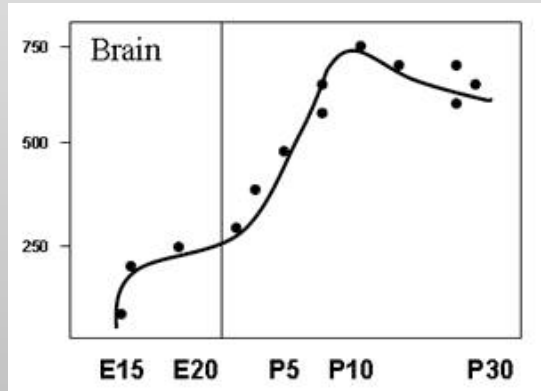


- **Thyroid Hormone (TH)**
- **Arylhydrocarbon Receptor (AhR)**
- **Valproic Acid**
 - **Histone Deacetylase (HDAC) inhibition**
 - **Apoptosis**
- **HCA (Cellomics Arrayscan & self-written algorithms 'Omnisphero')**

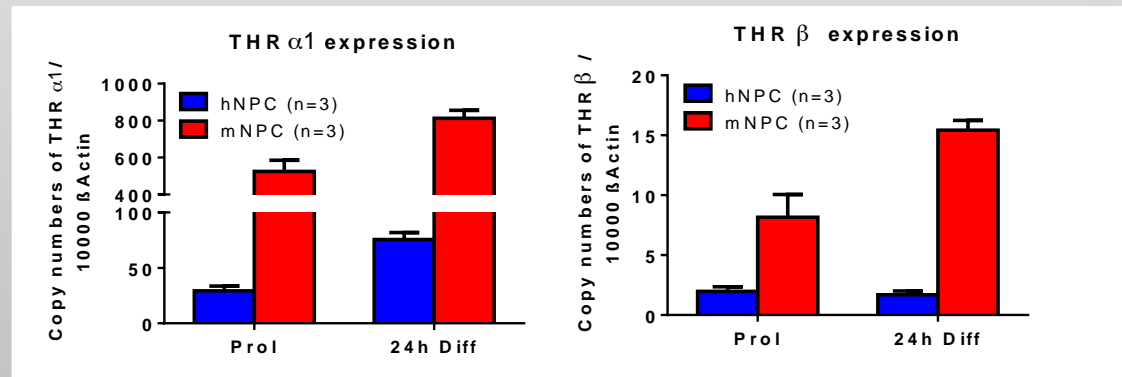
Thyroid Hormone



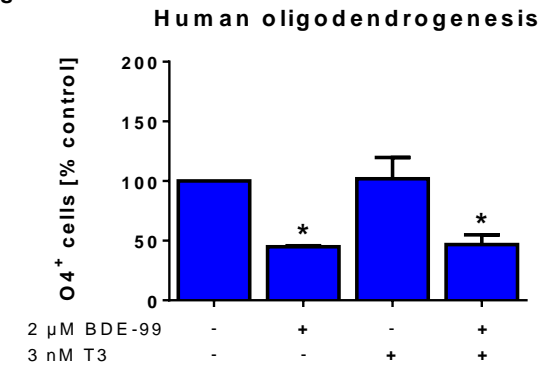
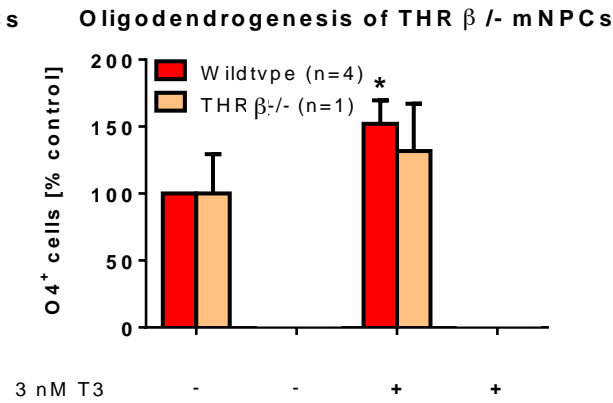
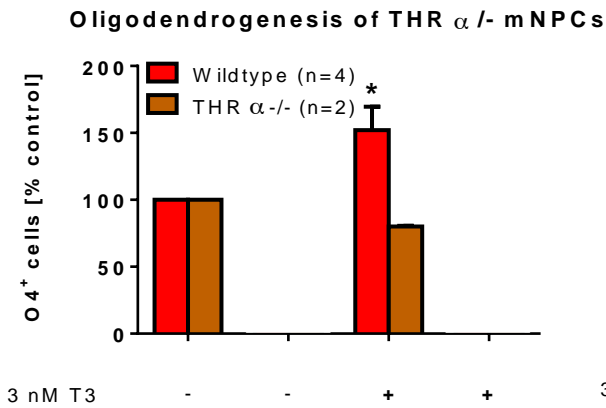
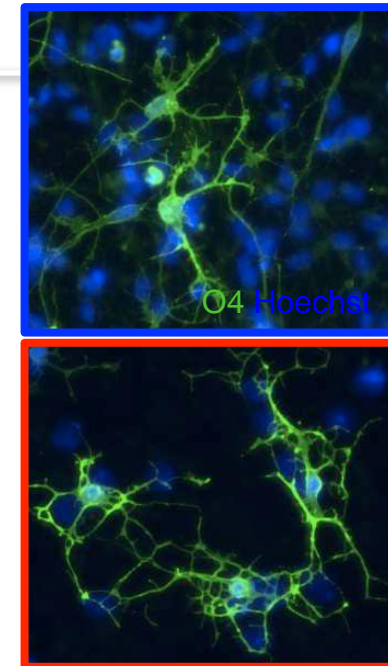
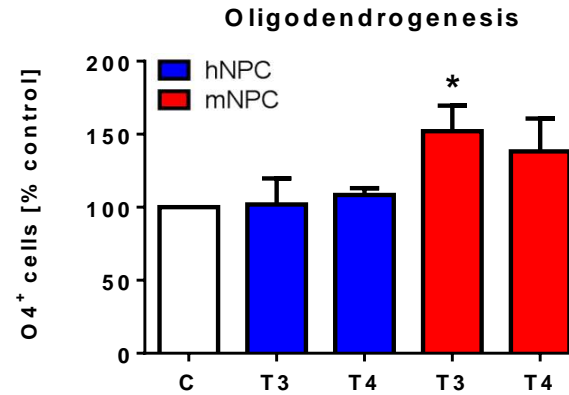
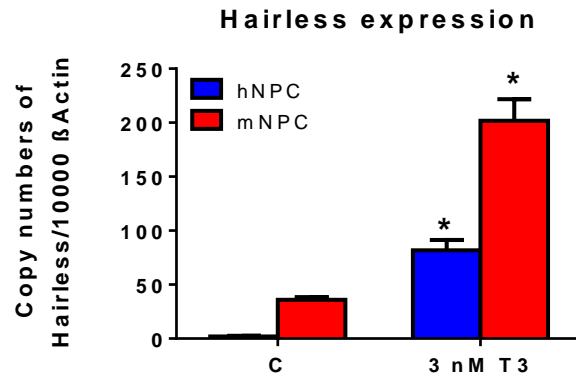
Ontogeny of T3 receptors in rat



Perez-Castillo et al, Endocrinology
117:2457-2461,1985

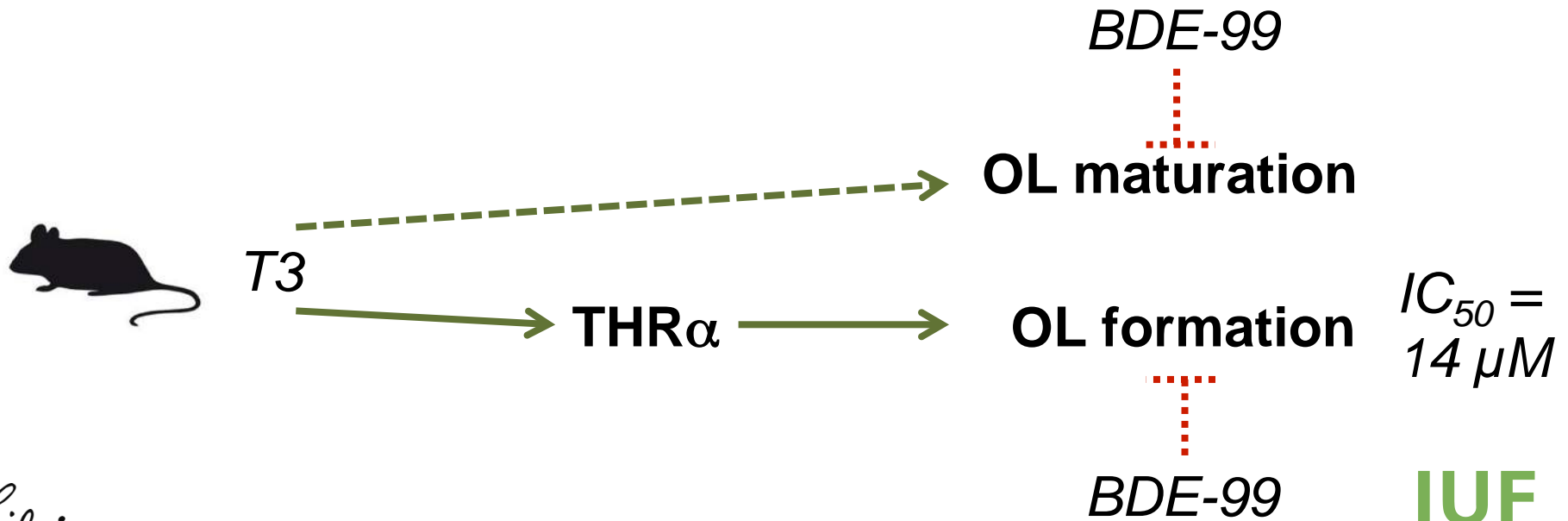
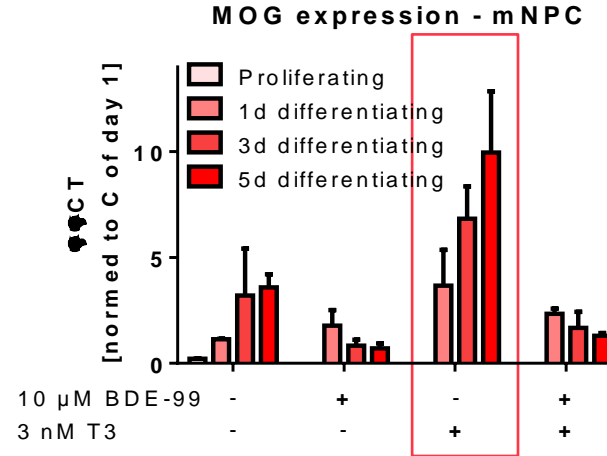
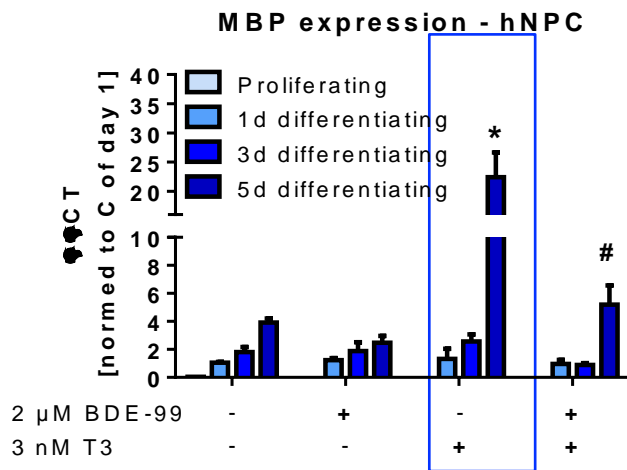


Thyroid Hormone

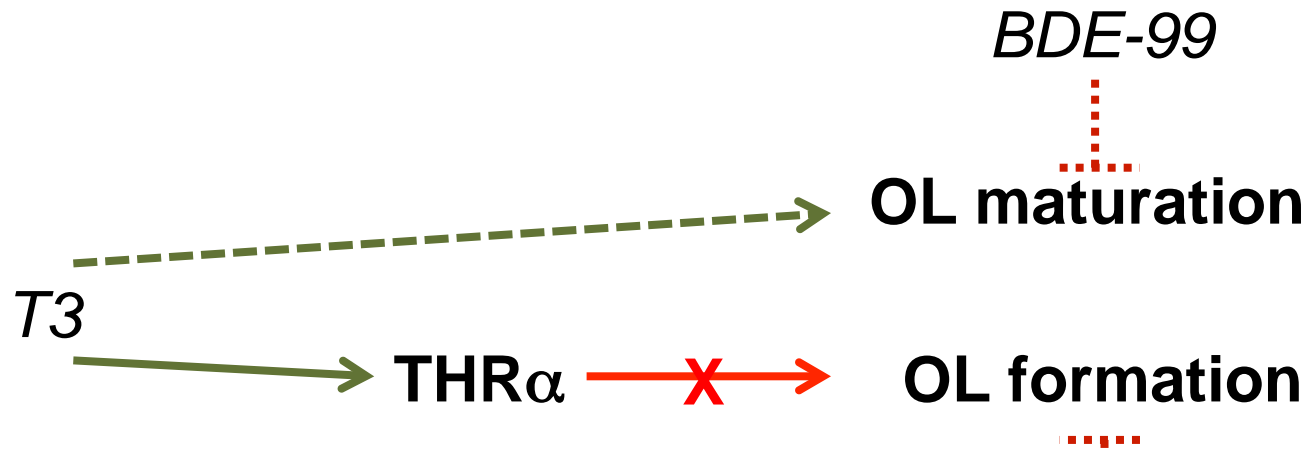
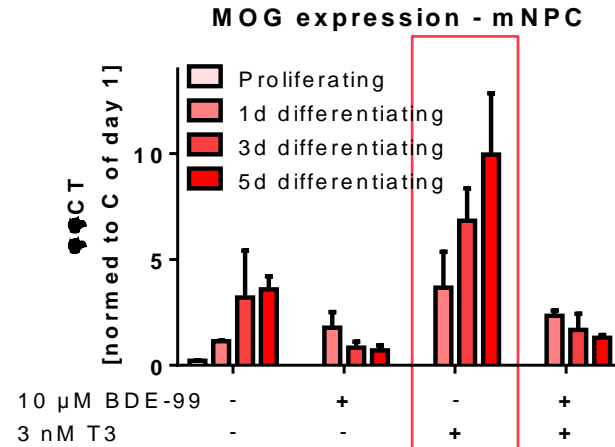
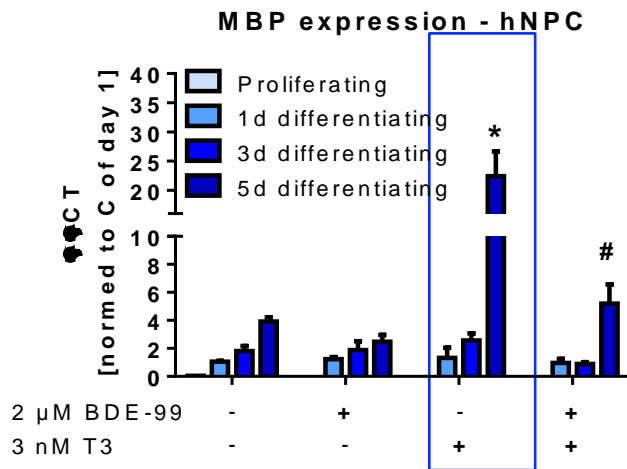


Transgenic animals kindly provided by Heike Heuer, IUF

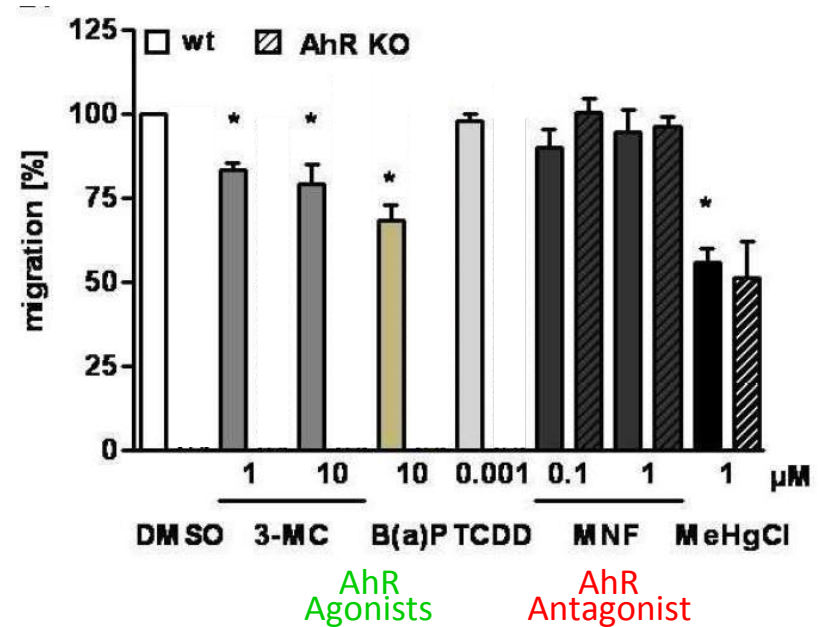
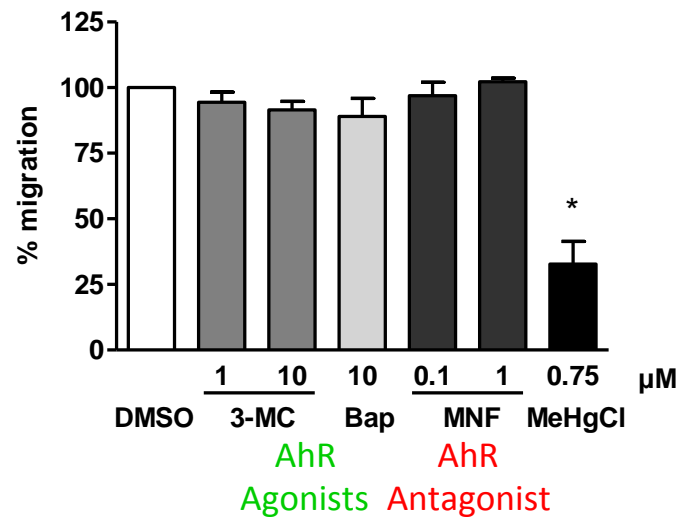
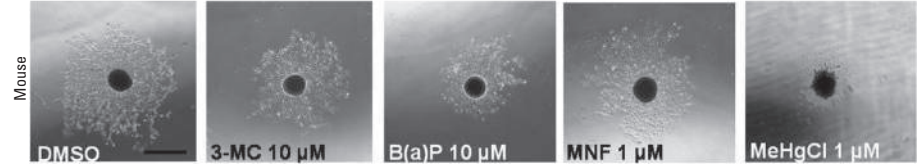
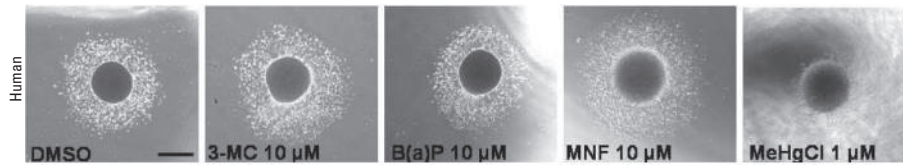
Thyroid Hormone



Thyroid Hormone

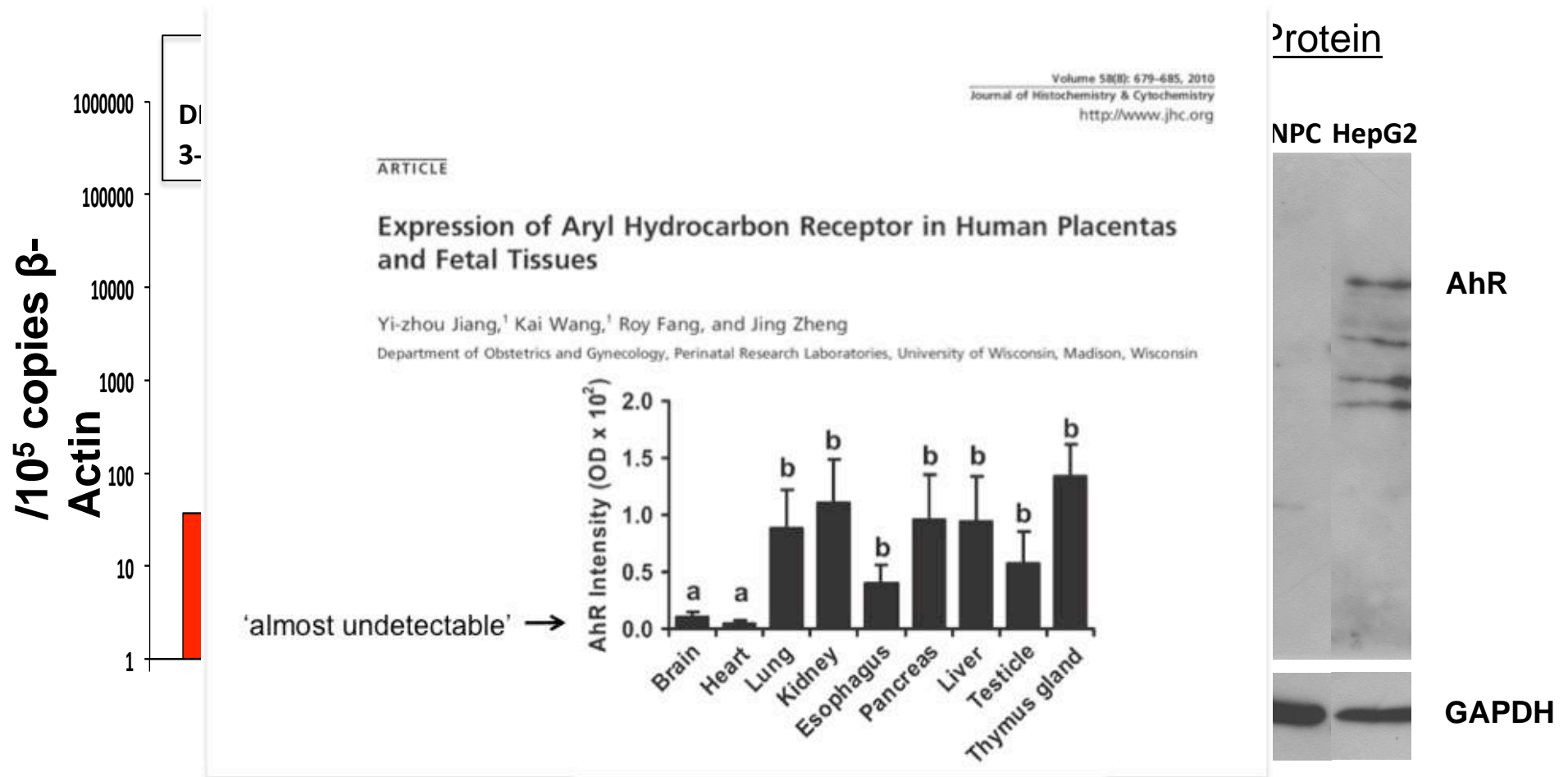


AhR - migration



Gassmann et al. Environ Health Perspect 2010

AhR - gene/protein expression



Human NPC are protected against AhR-dependent toxicity of PAH due to lack of AhR expression

Valproic Acid



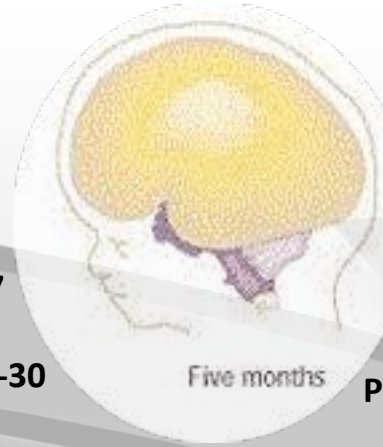
35 days

E11-E14
=
GW 6-9



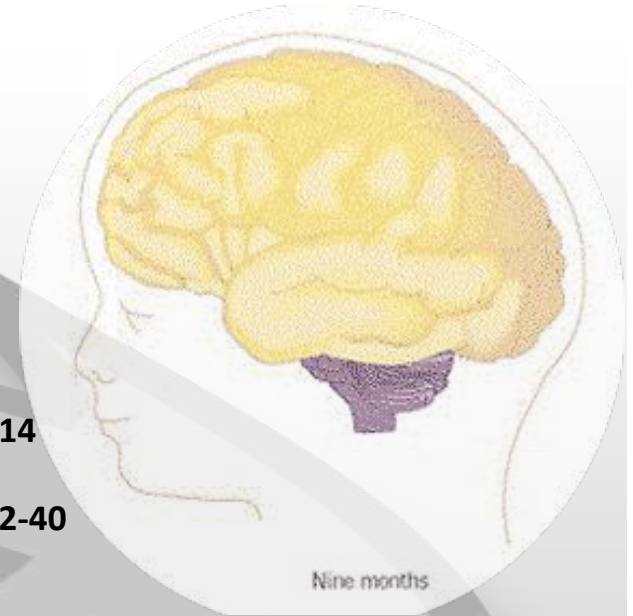
100 days

P1-7
=
GW 18-30

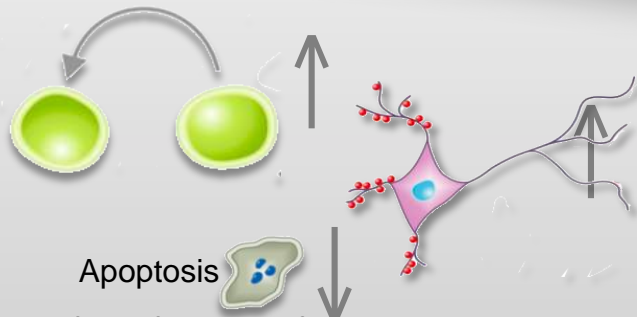


Five months

P8-14
=
GW 32-40



Nine months



Apoptosis

Molecular mechanisms:

Proliferation:

wnt – β -catenin (Foti et al. 2013)

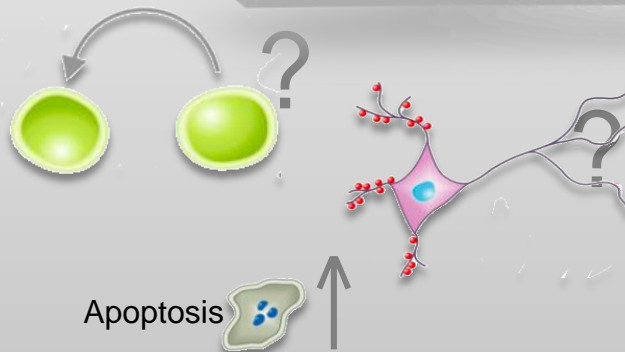
Apoptosis:

Bcl-2 expression (Go et al. 2011)

Differentiation:

suggested indirectly by
increasing the NPC pool?

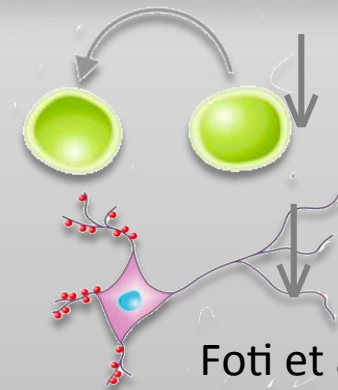
HDAC inhibition suggested
(Montgomery et al. 2004)



Apoptosis

Apoptosis:

Bcl-2 expression (Go et al. 2011)



Foti et al. 2013

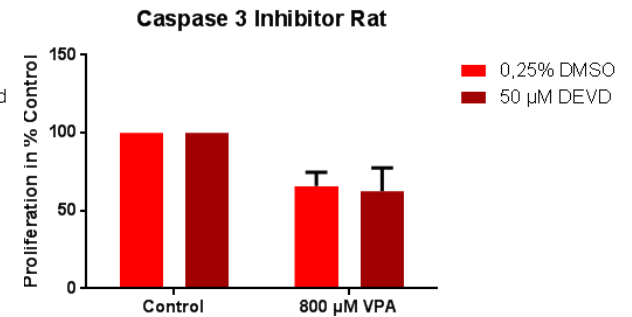
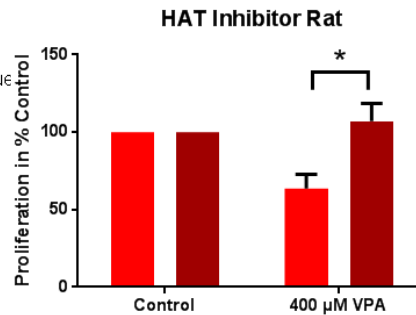
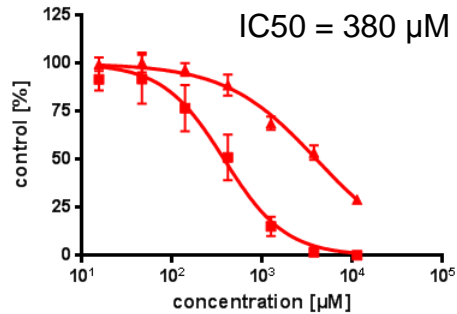
Mech.: HDAC inhibition

Apoptosis

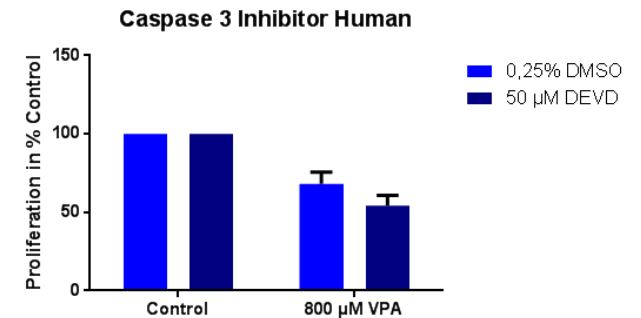
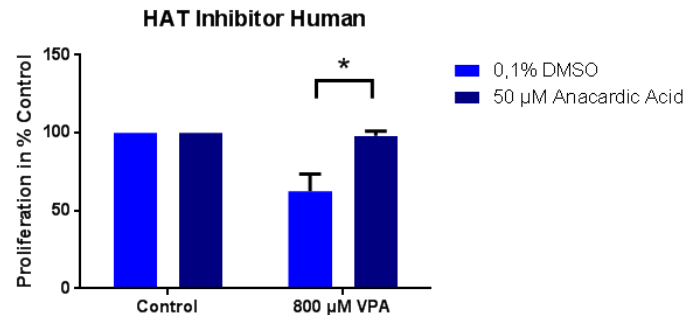
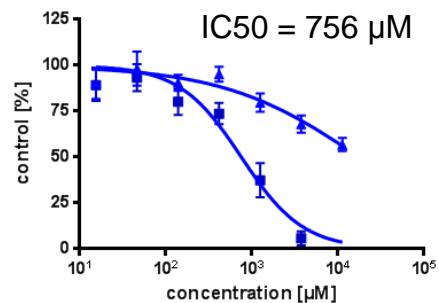
Yochum et al. 2011

Valproic Acid - NPC proliferation

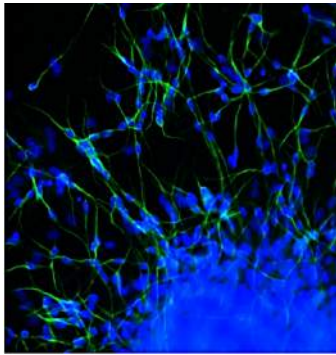
RAT



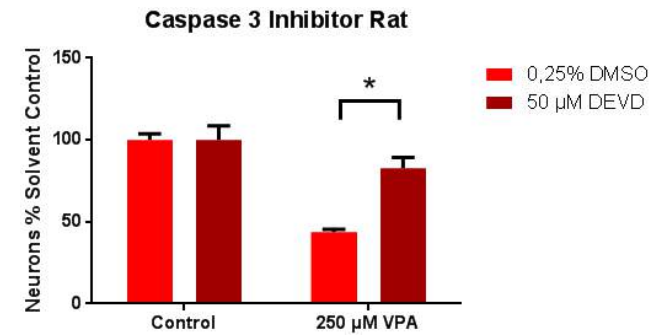
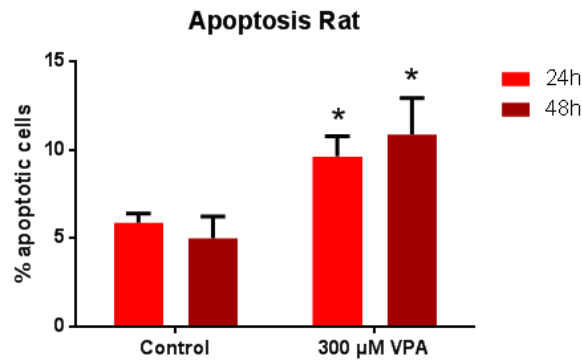
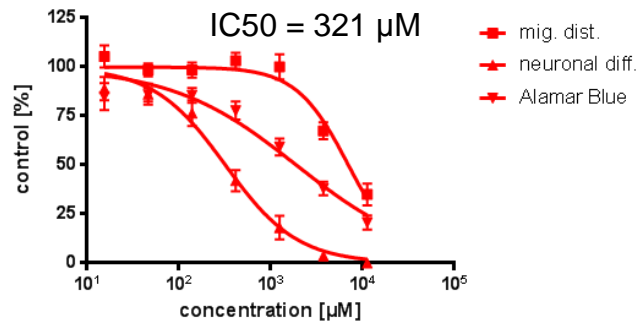
HUMAN



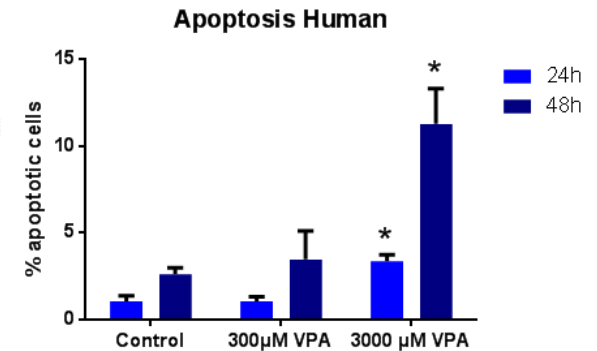
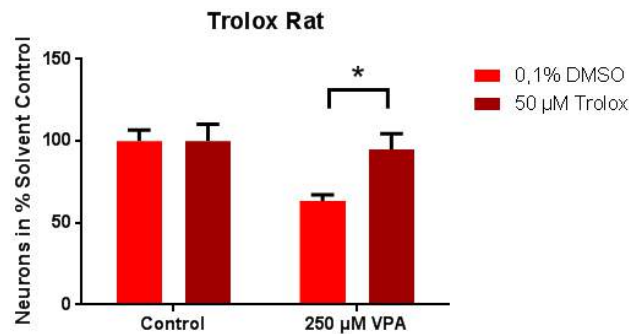
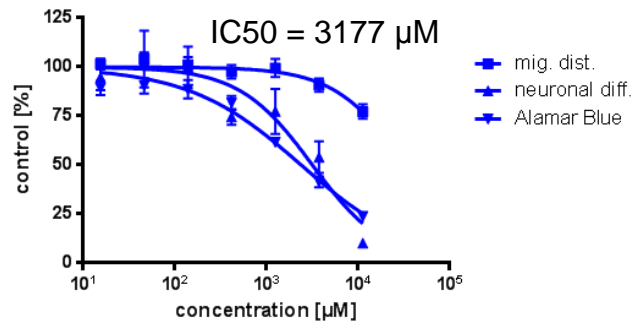
Valproic Acid- NPC differentiation



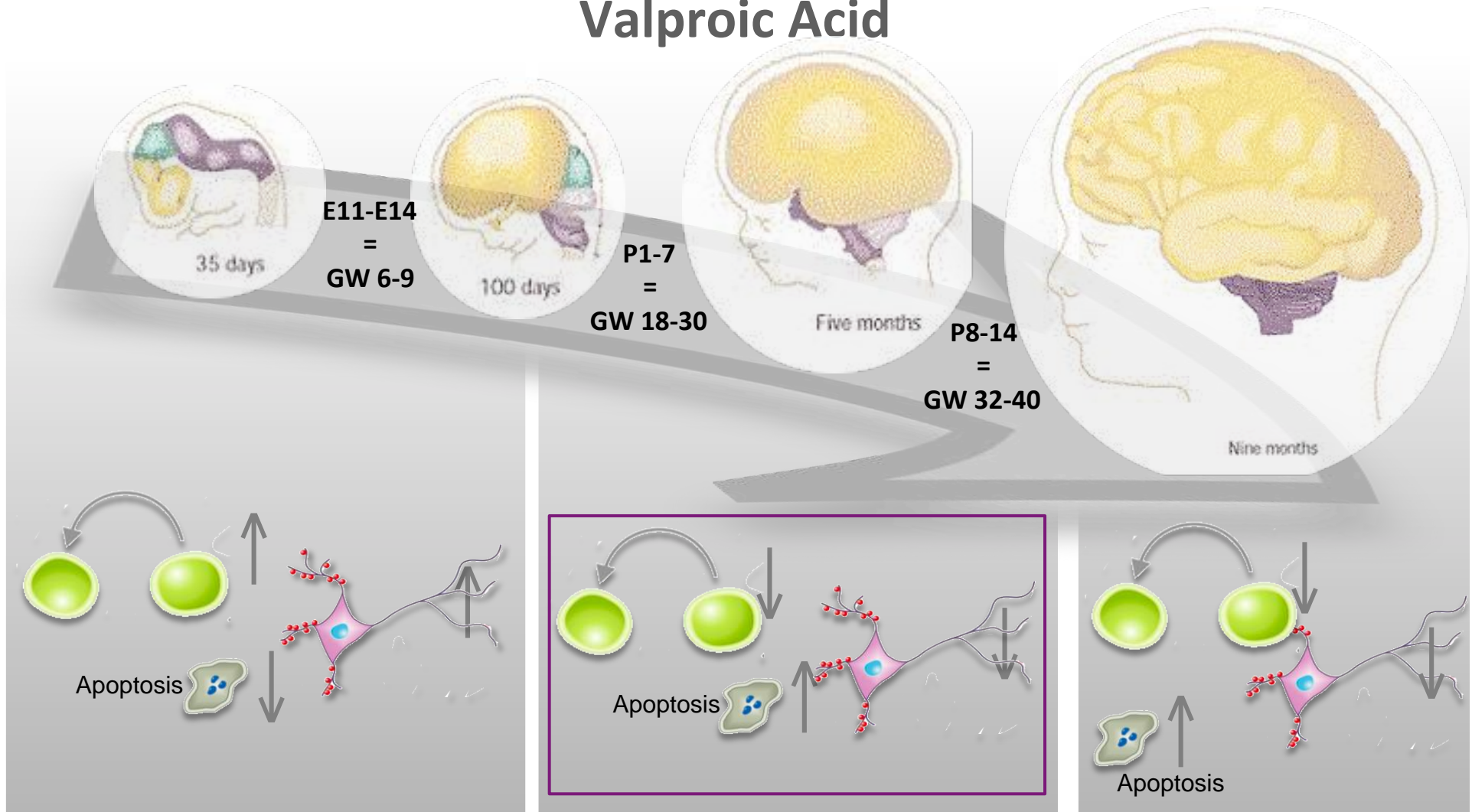
RAT



HUMAN



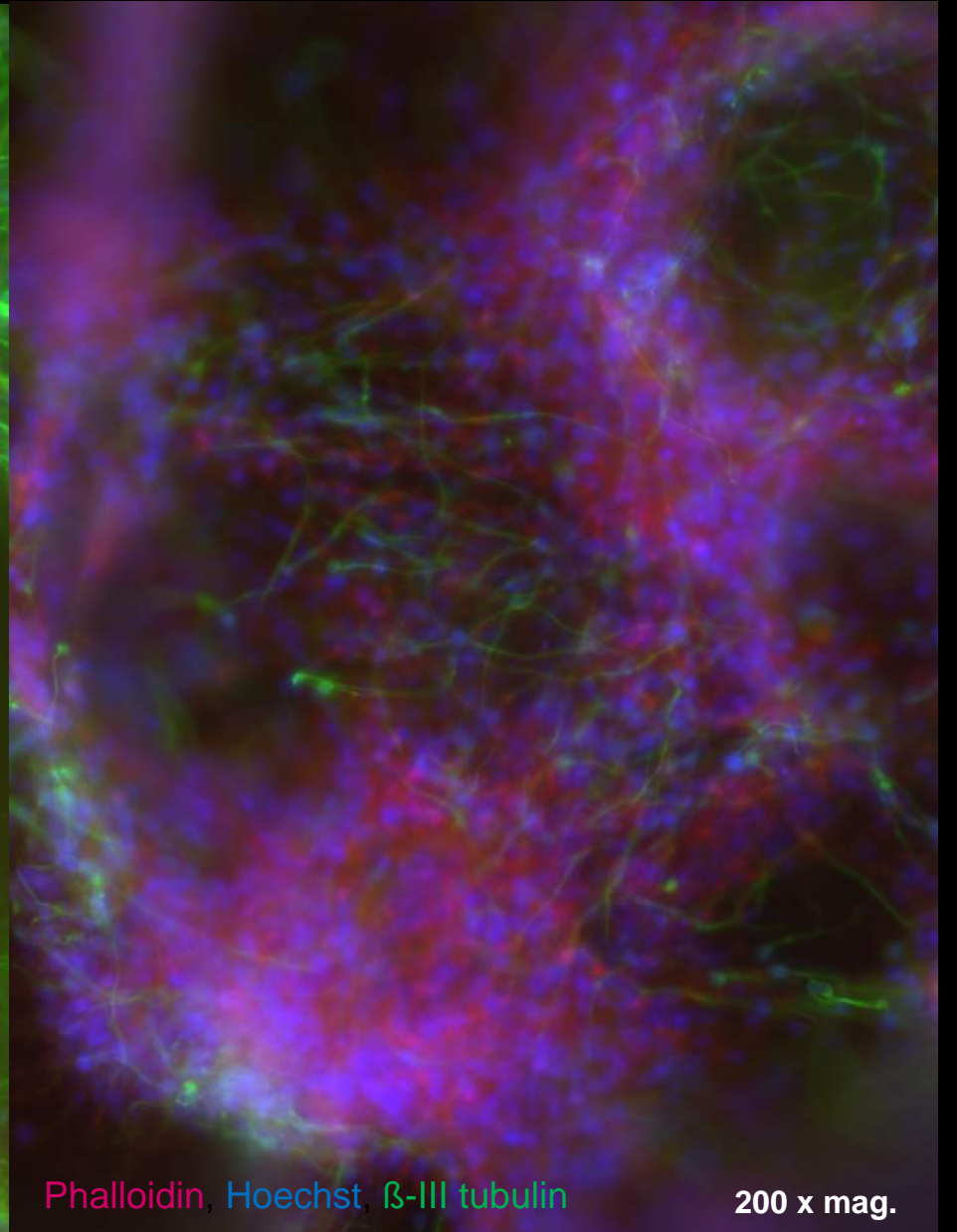
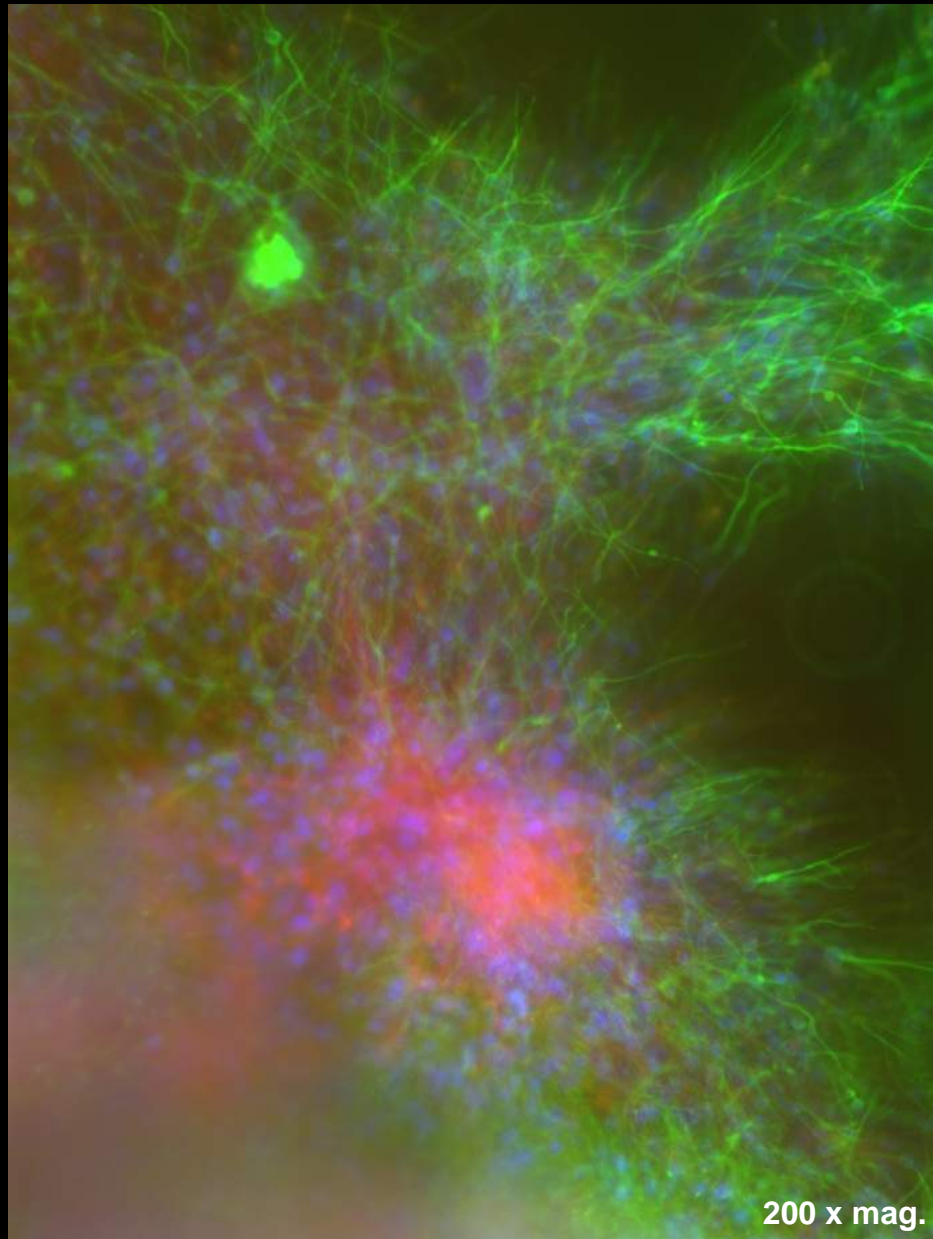
Valproic Acid



- NPC proliferation is inhibited by VPA-dependent HDAC inhibition (Baumann et al. in prep)
- VPA does not inhibit neuronal differentiation of rat NPC, but induces neuronal apoptosis due to formation of ROS.
- Human neurons are protected against VPA-induced apoptosis.

Long-Term Culture (25d) of hNPCs in Mal-PVA Hydrogels (Cellendis)

(Hellwig et al. in preparation)

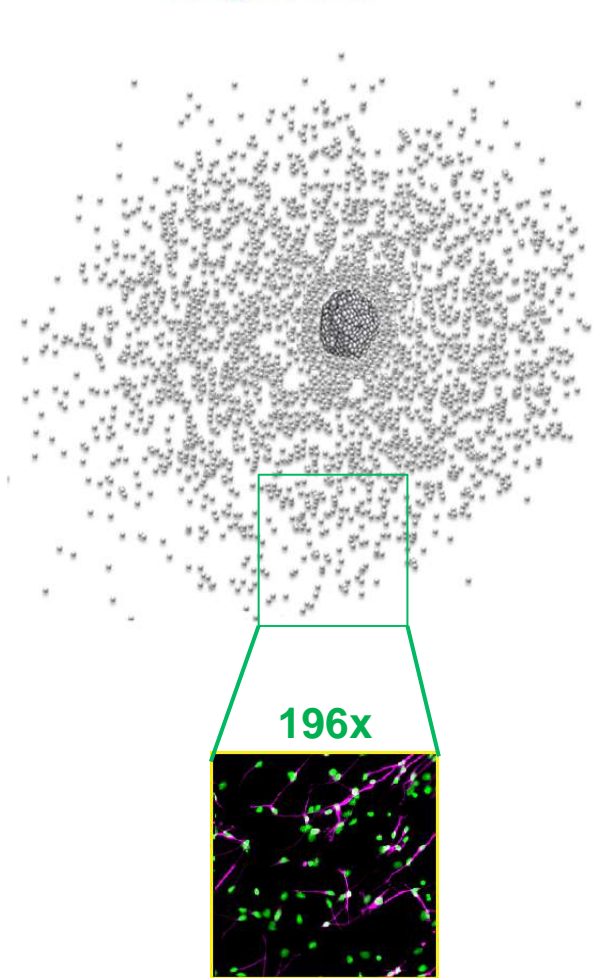


Summary (I)

- **Primary Neurospheres seem to conserve NPC molecular signatures and functions *ex vivo* into *in vitro*.**
- **Due to the multiple neurodevelopmental processes neurospheres are apt to mimic *in vitro*, they are well suited for investigations ‘from pathway to function’.**
- **A variety of interspecies differences in NPC signaling seem to exist between human and rodent NPCs.**
- **Studying the molecular similarities/differences of neurospheres will contribute to human risk assessment for DNT, especially in the context of the ‘Adverse Outcome Pathway’ concept.**

HCA in differentiating NPC

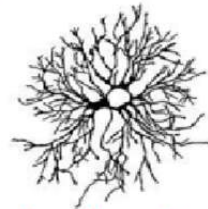
Migration



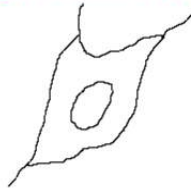
Differentiation



Neurons



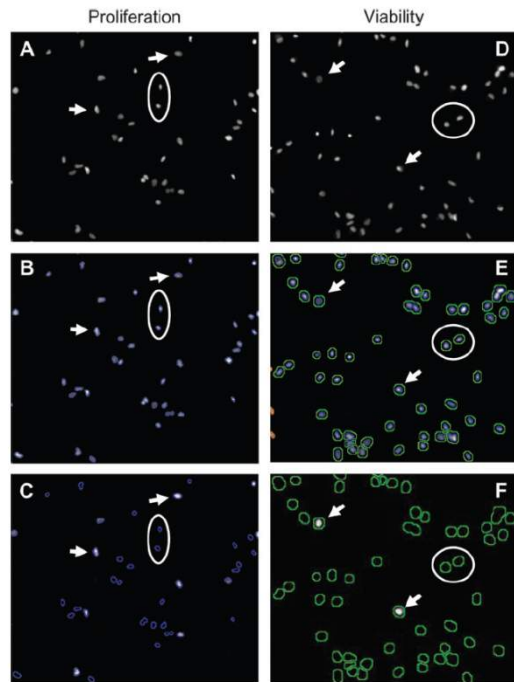
Oligodendrocytes



Astrocytes

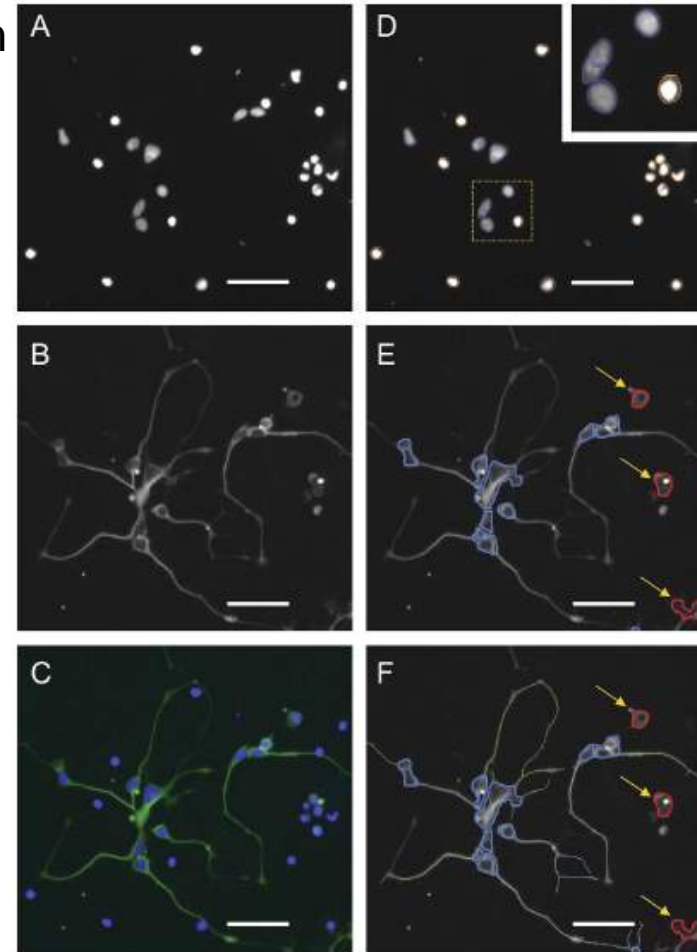
- Information on the whole migration area, not just random areas of a well.
- Software needs to distinguish between different cell types.
- Issue of a high density culture needs to be overcome and neurons correctly identified.
- Varying densities in different areas around sphere core.

High Content Image Analyses (HCA) for DNT testing

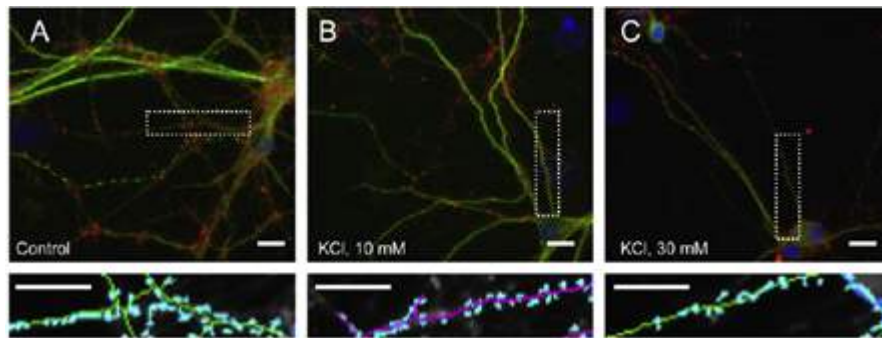


Proliferation/
Viability

Neurite Outgrowth



Synaptogenesis



Leibniz

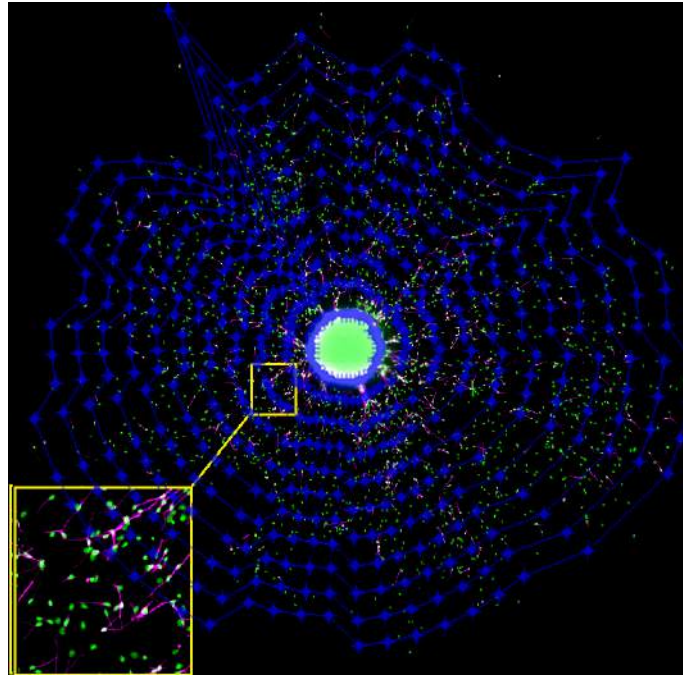
From: Breier et al., *Toxicological Science*, 2008
Harrill et al., *Neuro Toxicology*, 2010
Harrill et al., *Toxicology in vitro*, 2011

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HCA in differentiating NPC

Omnisphero



Neuron/Nuclei Identification

Neuronal Quantification

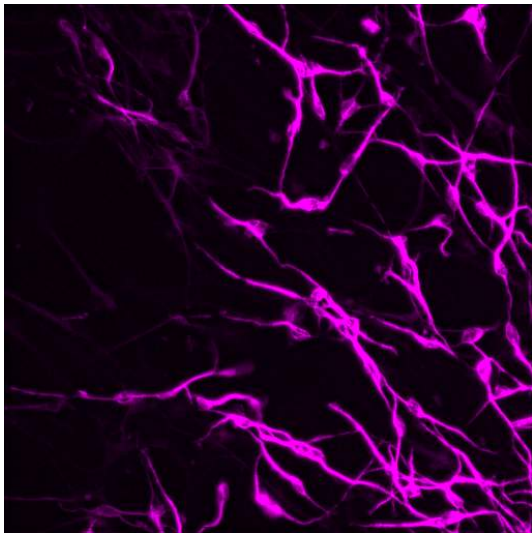
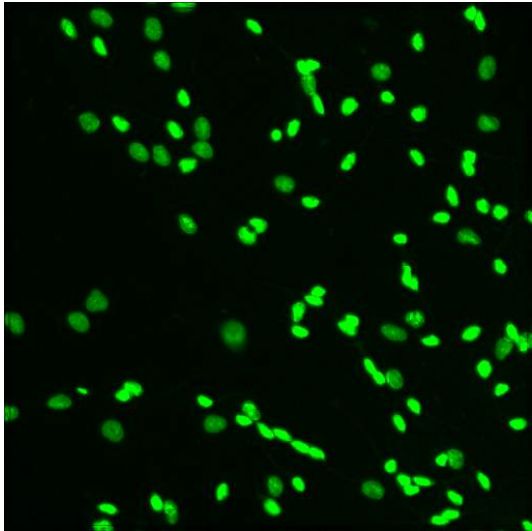
Neuronal Morphology

Migration

Neuronal Positioning

Omnisphero: Image Pre-processing

Raw images

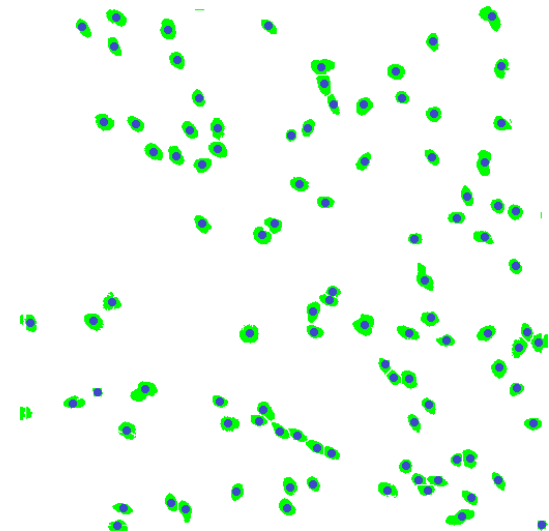


Thresholding (Isodata)

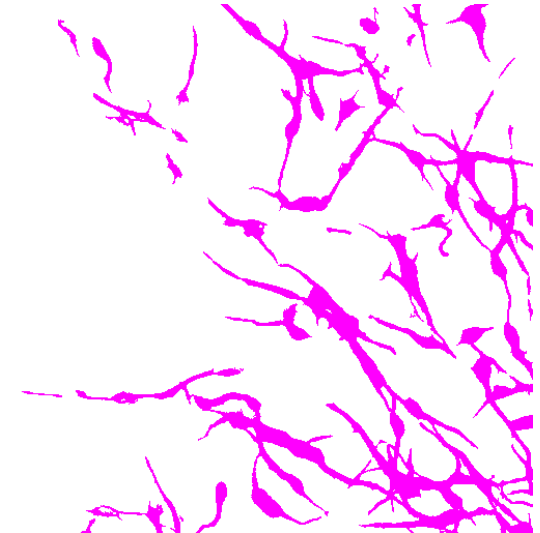


Watershade

Binary images



Thresholding (Isodata)

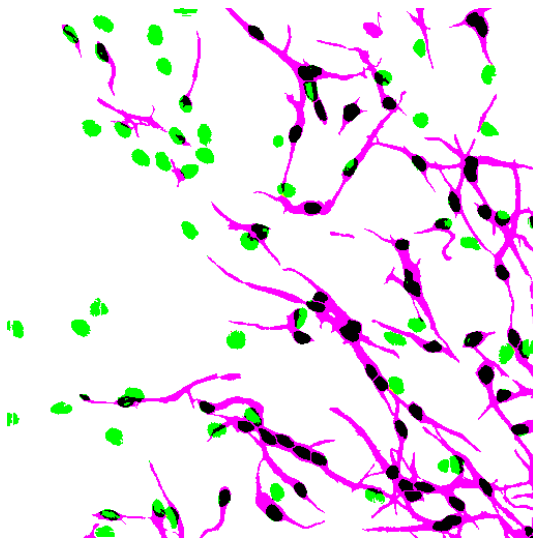


Leibniz

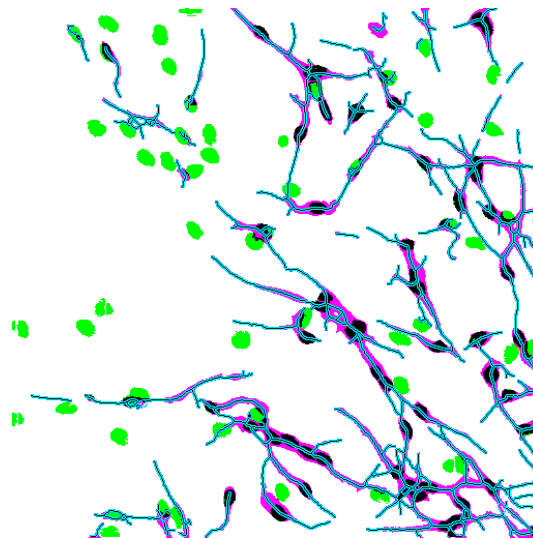
Schmuck et al. in preparation

Omnisphero: Neuron Identification

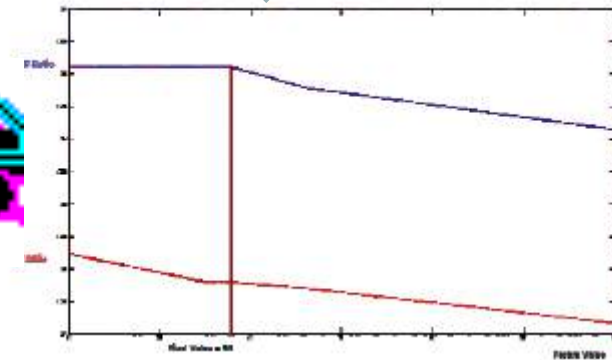
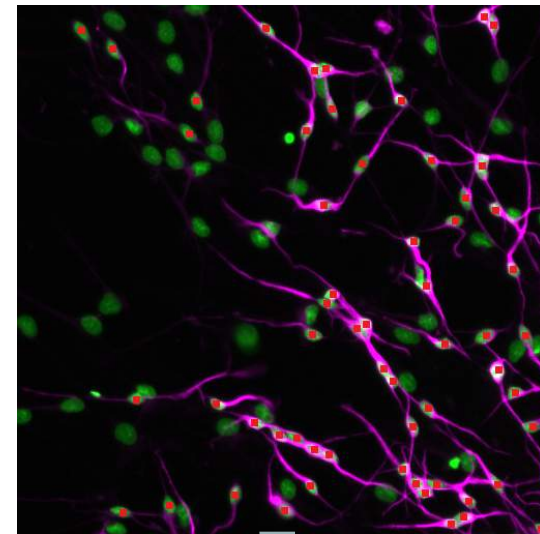
Composite Fill



Neuron Tracer



Optional: Manual annotation



Leibniz

Schmuck et al. in preparation

Omnisphero: Automated Analyses

The screenshot displays the Omnisphero software interface, titled "<Student Version> : Omnisphero". The interface includes a menu bar (File, Operations, Algorithms, Options) and several configuration panels on the left. The central area shows a microscopy image of a cell with a large green nucleus and numerous red puncta. The right side features a table with columns for Well, Filter ID, and #Nuclei Ce. Below the table are input fields for Edge length a (Pixels) and Edge length b (Pixels). The bottom left shows a Pixel info field (X, Y) [R G B].

Snail Version

- 48 Well Plate 8 Bit
- 48 Well Plate 16 Bit
- 96 Well Plate 8 Bit 484 Pics
- 96 Well Plate 8 Bit
- 96 Well Plate 16 Bit
- OT Single Chamber 8 Bit
- OT Single Chamber 16 Bit
- Whole OT 8 Bit
- Whole OT 16 Bit

GUI

- Nucleus Picture Show Rings
- Neurite Picture Binary Pic
- Skeleton Picture Remove Core

Show Points

Skeleton Ne... Minus

Manual Mode

- Main Manual 1 Manual 3
- Manual 2 Manual 4

Well

E7
E8
E9
F10
F3

Filter

Filter Type

- Rectangles
- Polygon
- Squares
- Auto
- Negative

Zoom in

Preview Preview Auto

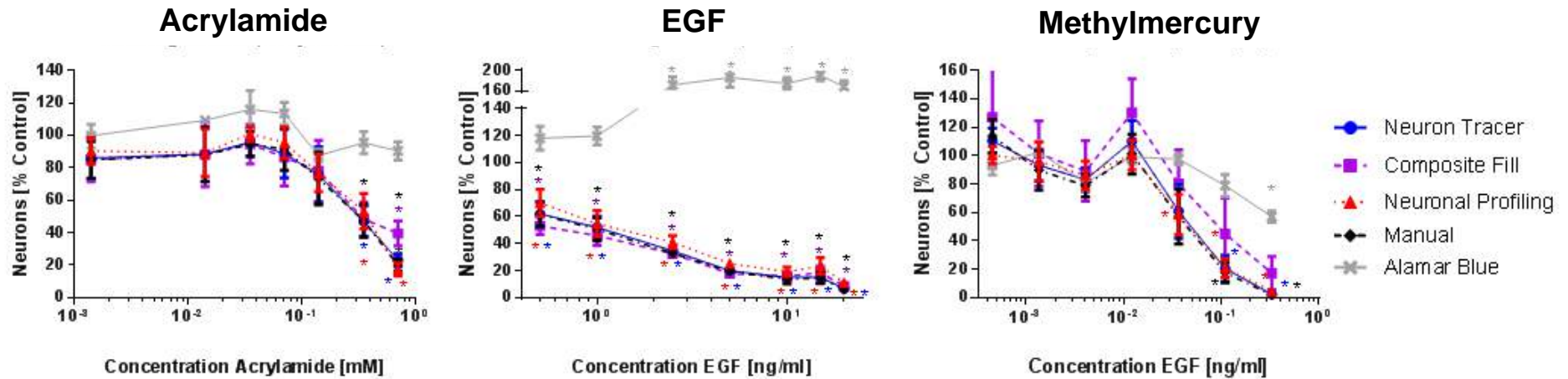
Well	Filter ID	#Nuclei Ce
------	-----------	------------

Edge length a (Pixels)

Edge length b (Pixels)

Pixel info: (X, Y) [R G B]

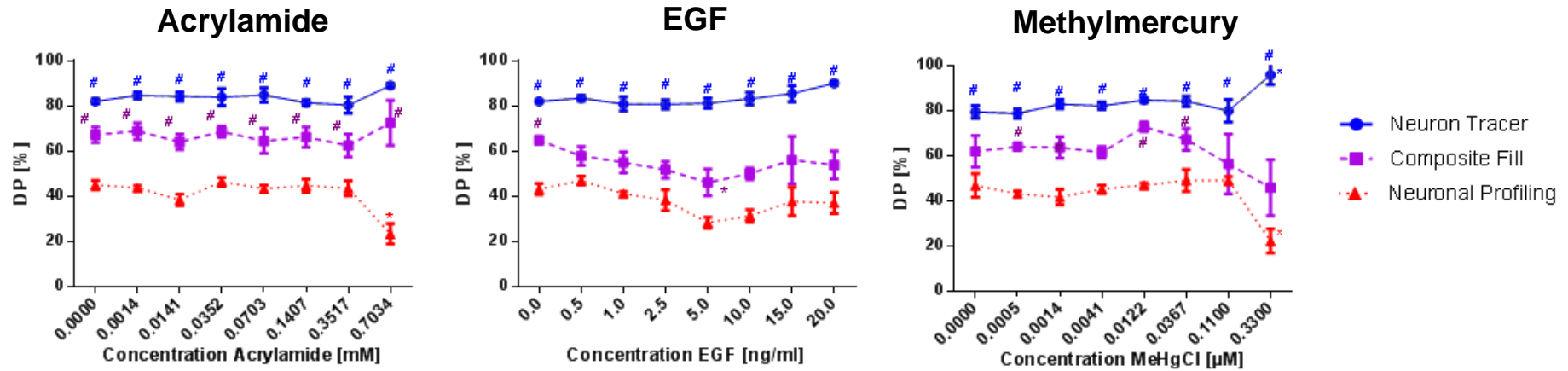
Neurogenesis: comparison of methods (IC₅₀ values)



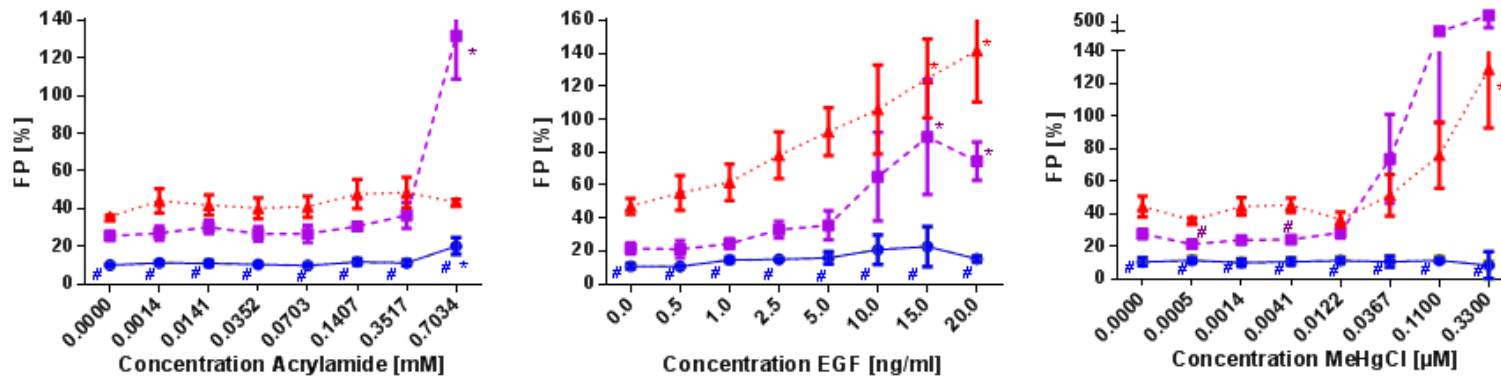
EC ₅₀ -Values	EGF [ng/ml]	Acrylamide [mM]	MeHgCl [μM]
Manual	0,96	0,30	0,045
Neuronal Profiling	1,442	0,34	0,049
Composite Fill	0,67	0,31	0,104
Neuron Tracer	1,022	0,41	0,051

Neurogenesis: Accuracy & Precision

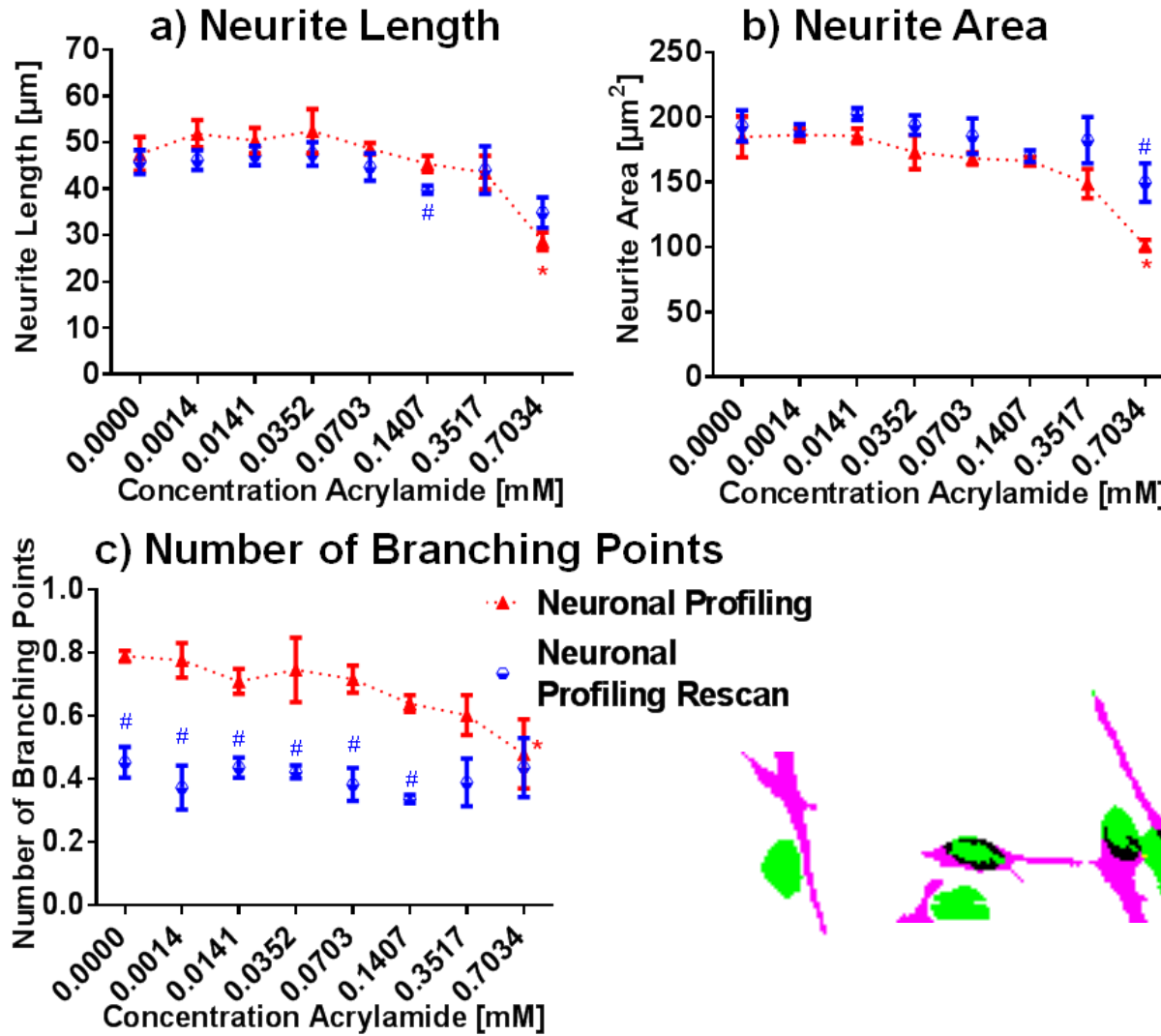
Detection Power (DP)



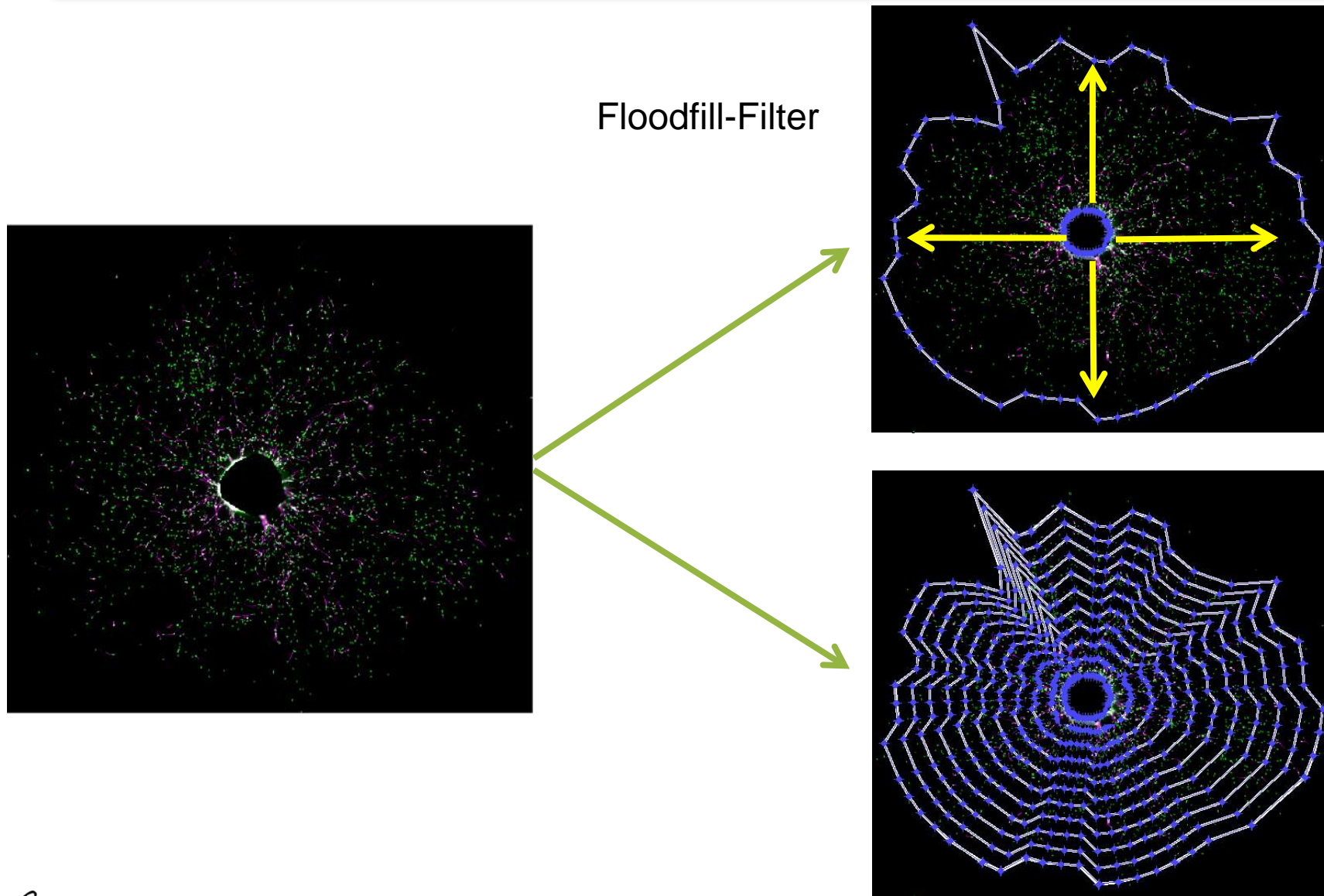
False-Positives (FP)



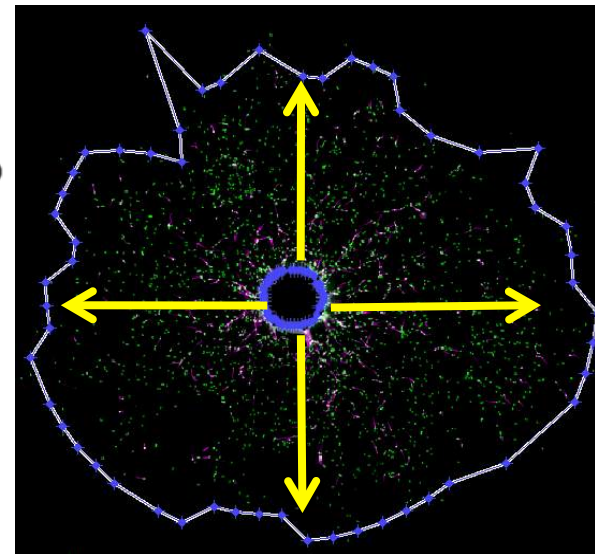
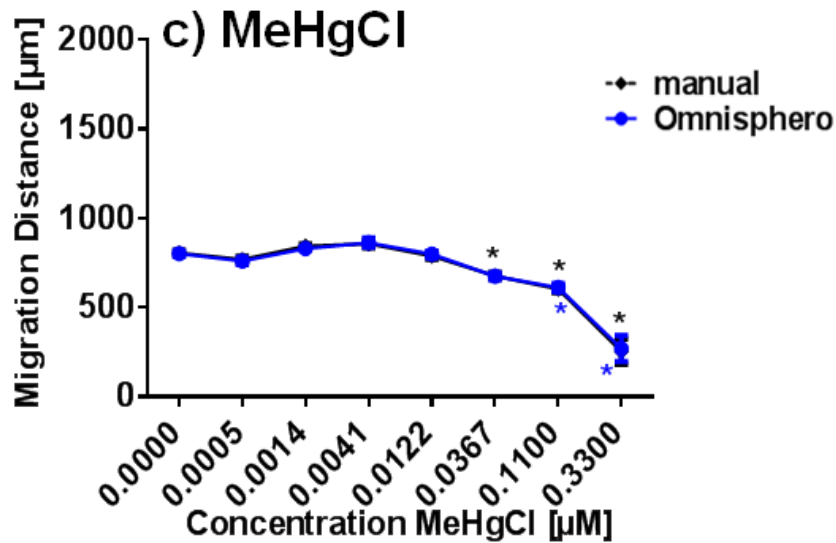
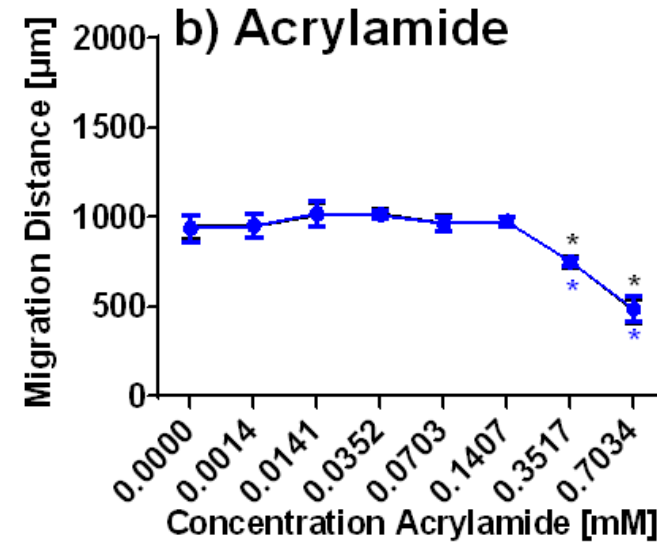
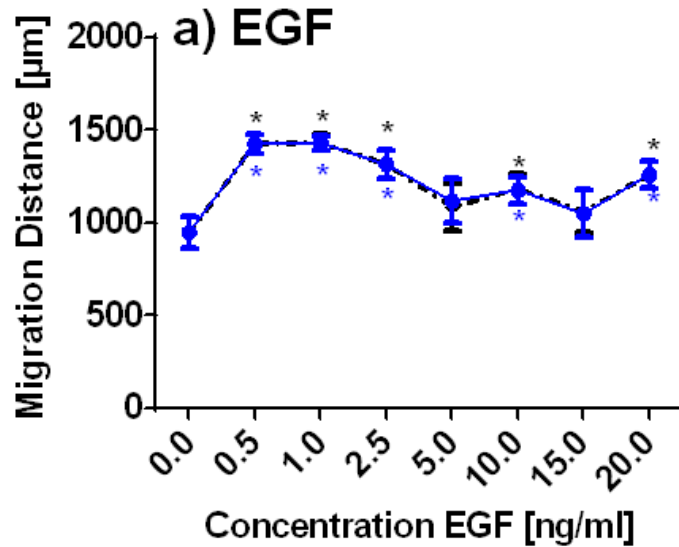
Neurite outgrowth: Accuracy & Precision



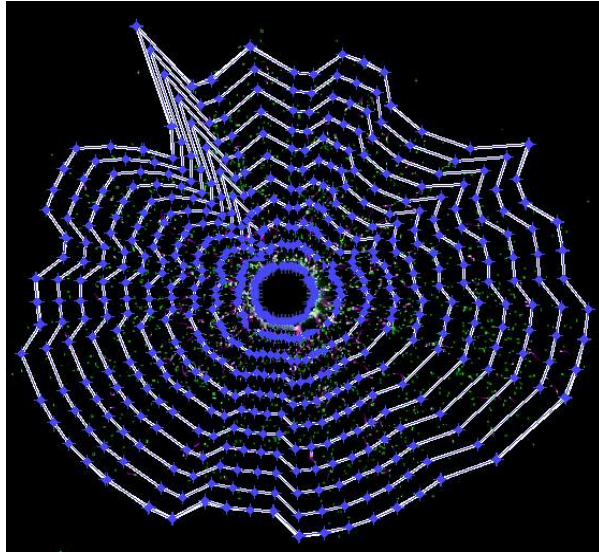
Sphere-specific Endpoints



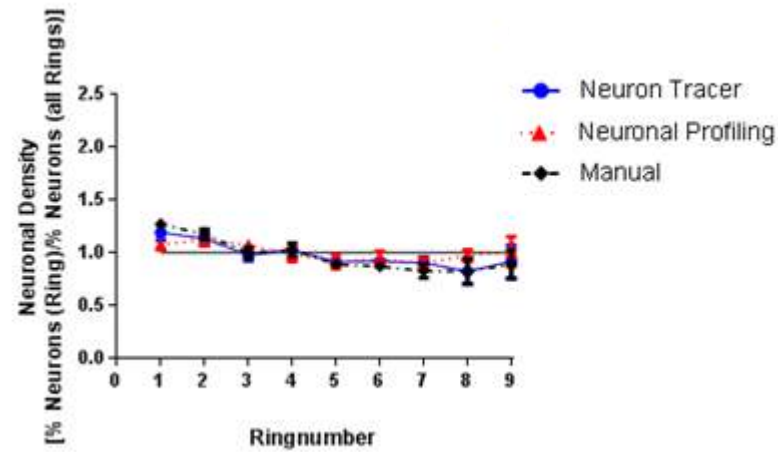
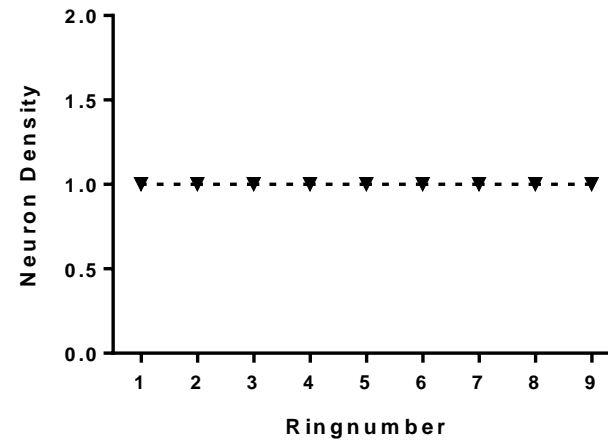
Sphere-specific Endpoints: Migration distance



Neuronal Density Distribution

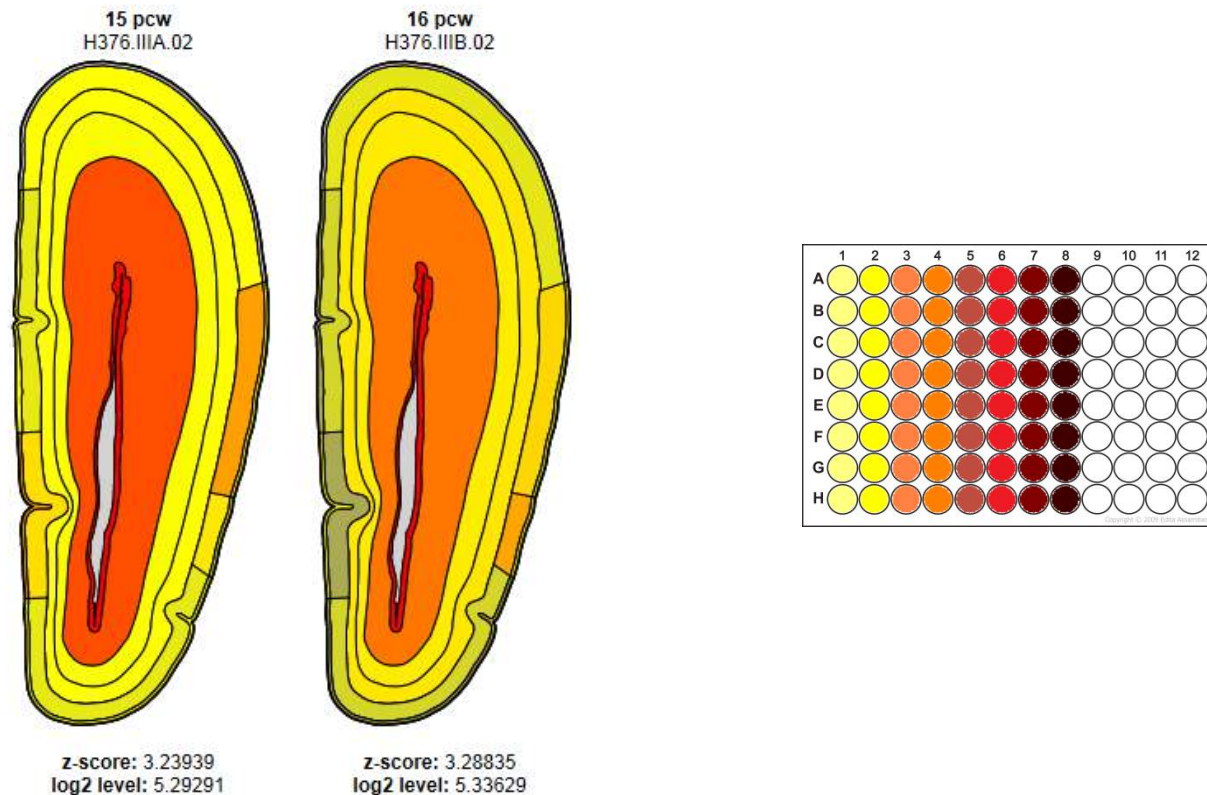


$$\sigma_n = \frac{\frac{n_n(\text{Neurons})}{n_n(\text{Nuclei})}}{\sum_1^n \frac{n_n(\text{Neurons})}{n_n(\text{Nuclei})}}$$

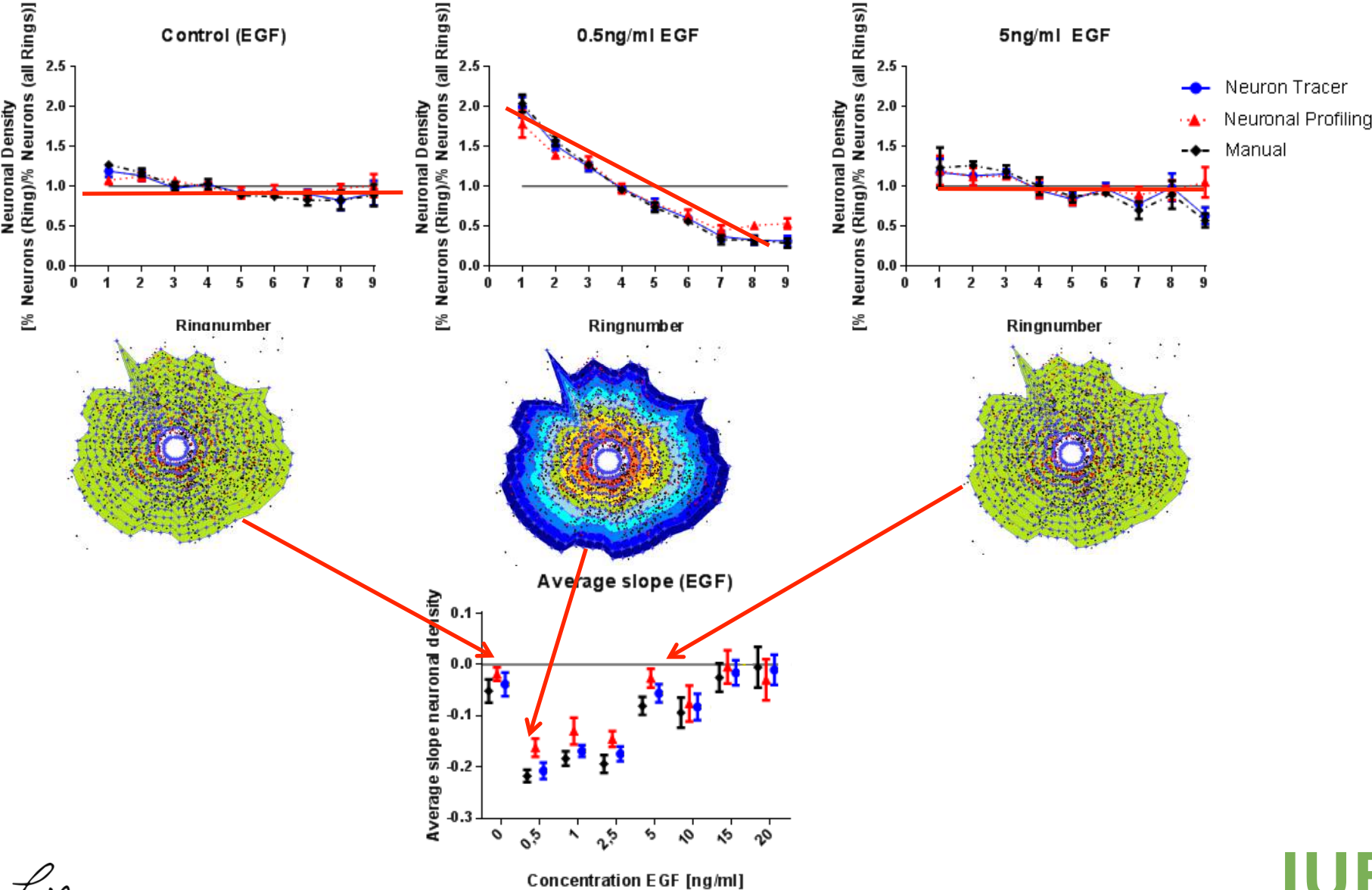


EGF transactivation of Trk receptors regulates the migration of newborn cortical neurons

Dirk Puehringer¹, Nadiya Orel¹, Patrick Lüningschrör¹, Narayan Subramanian¹, Thomas Herrmann¹, Moses V Chao² & Michael Sendtner¹

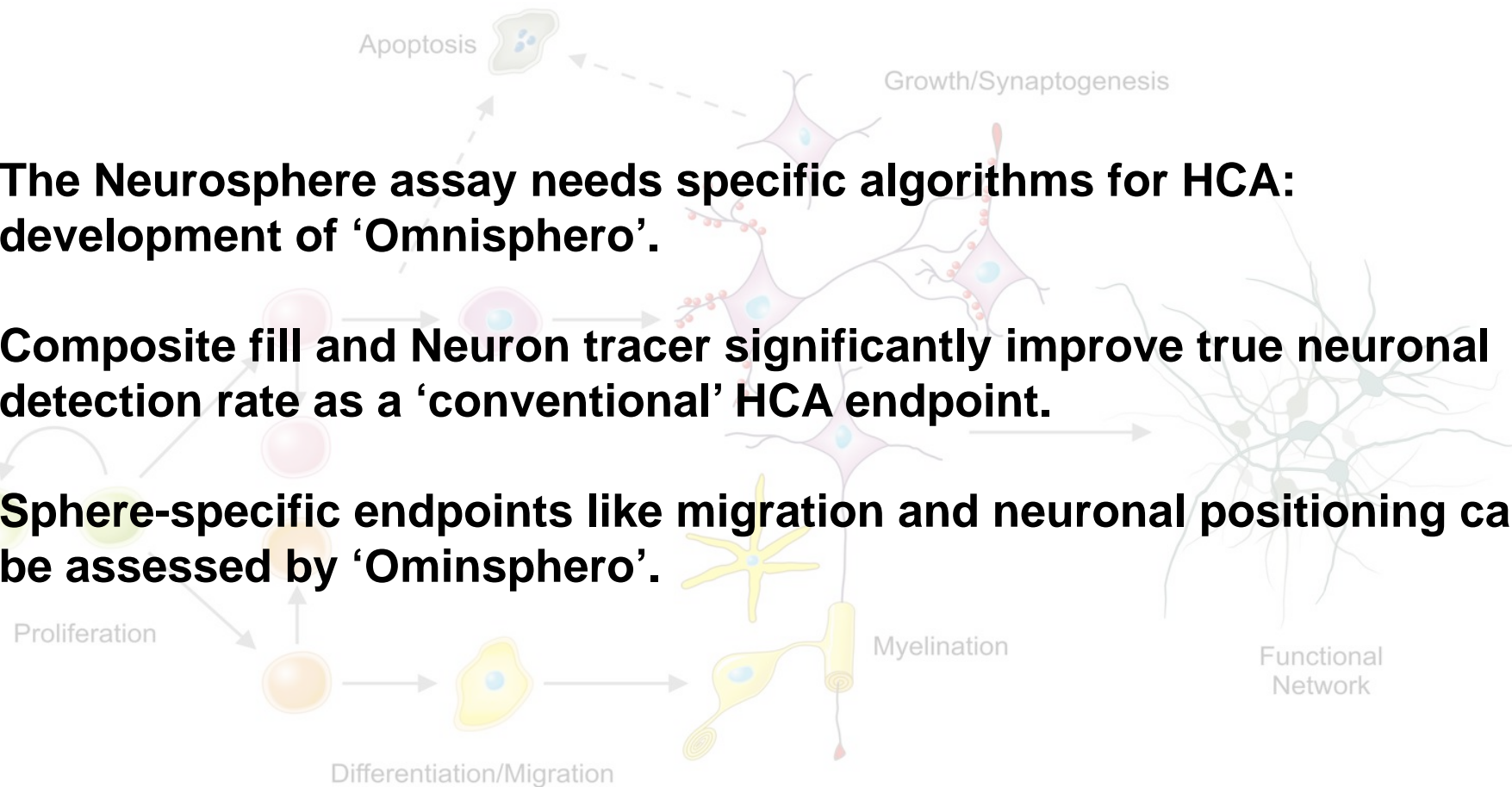


Neuronal Density Distribution



Summary (II)

- **The Neurosphere assay needs specific algorithms for HCA: development of ‘Omnisphere’.**
- **Composite fill and Neuron tracer significantly improve true neuronal detection rate as a ‘conventional’ HCA endpoint.**
- **Sphere-specific endpoints like migration and neuronal positioning can be assessed by ‘Ominsphero’.**



Acknowledgements

Dr. Marta Barenys
Dr. Julia Tigges
Dr. Susanne Giersiefer
Dr. Janette Schuwald
Dr. Henrik Alm
Jenny Baumann
Katharina Dach
Martin Schmuck
Maxi Hofrichter
Stefan Masjosthusmann
Christine Hellwig
Christiane Hohensee
Laura Nimtze
Denise de Boer
Ulrike Hübenthal

Cooperation partners:

Pamela Lein, UC Davis, USA
Heike Heuer, IUF, Düsseldorf
Axel Mosig, University of Bochum
Kai Stühler, HHU Düsseldorf



**German Alternative
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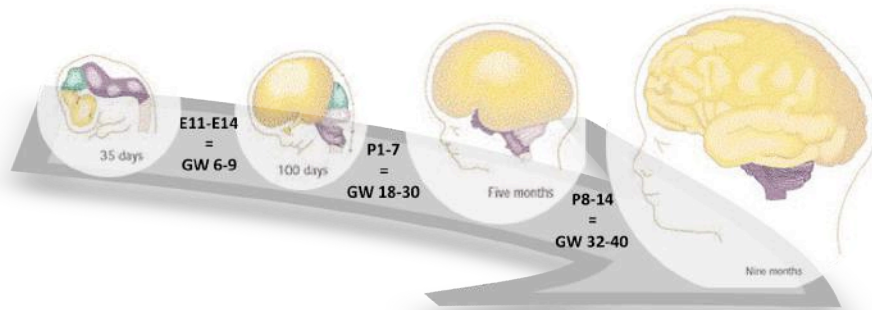
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DNT – Translating Time

TRANSLATING TIME *across developing mammalian brains*



Translate

Please select two species, specify the location and process type of the event, input the PC day you want translated from Species One to Species Two, and click the translate button.

Species 1
Human

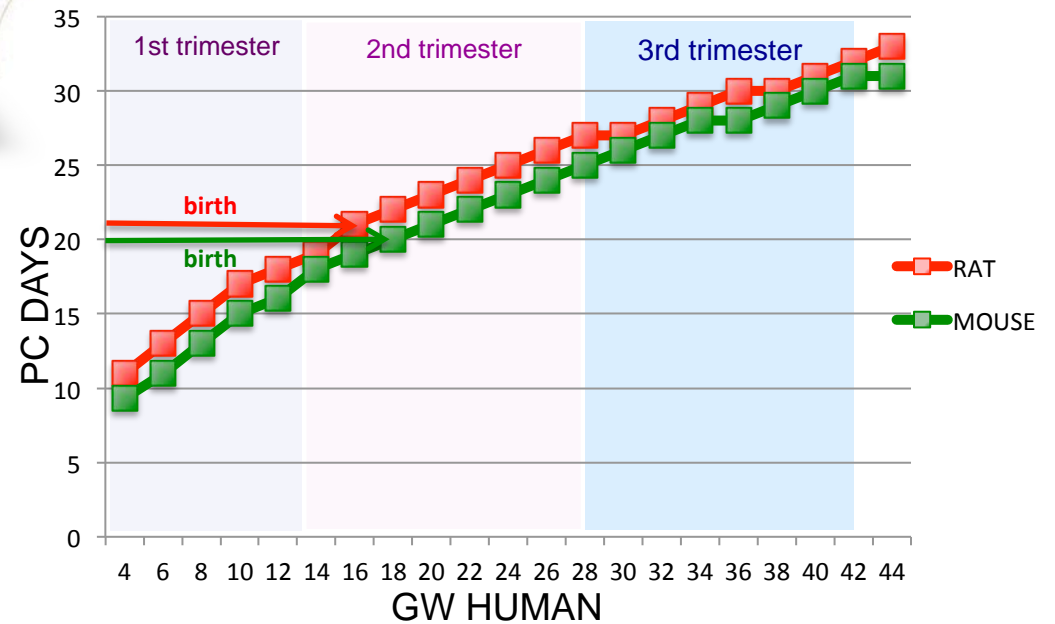
Process
Synapse Elimination and elimination

Location
Whole Brain

Days
24 < 140 < 979

Species 2
Rat

TRANSLATE



Processes: Brain Growth & Neurogenesis, whole brain

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Clancy et al. Neuroinformatics 2007

Nagarajan et al. Neuroinformatics 2010



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