EFSA conference: New approach methods (NAM) in toxicology for mechanism-based hazard assessment

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Chair inaugurated by the Doerenkamp-Zbinden Foundation,
University of Konstanz, Germany

Need of human cell-based models in toxicology

Predictivity

https://joshmitteldorf.scienceblog.com/category/uncategorized/

Mechanisms

?

Species barriers

https://www.innovativetesting.nl/news?page=3

Ressources, costs, throughput

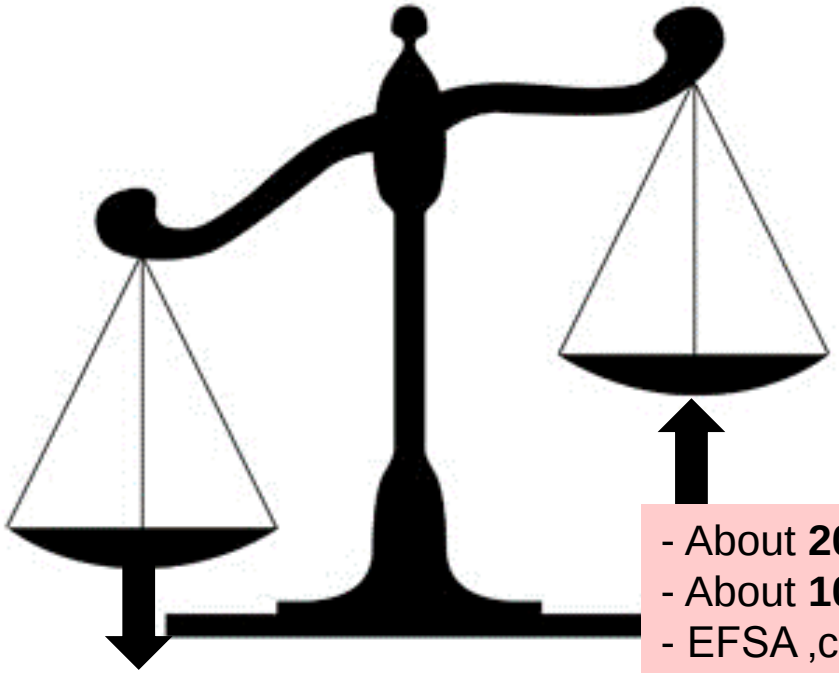
https://thedailyblog.co.nz/2015/07/10/money-as-a-social-technology/

Why not the good old way…?

fashion?

Why not the good old way…?

Why not the good old way…?



- About **200** regulatory DNT studies
- About **10** industrial chemicals
- EFSA ‚claims' 34 pesticides tested*
- Number of positives unclear (no survey)
- About **14** substances with human evidence

Reach: **> 8000** chemicals (high tonnage),
TSCA: **> 8000** high production vol. chemicals,
Hundreds of pesticides,
Thousands of food additives,
etc.…

** (unpublished)*

Two reasons to consider mechanisms

I. Making sense of data

II. Generation of data

Two reasons to consider mechanisms

I. Making sense of data → Examples

A. Animal studies

Aa: eye opening delayed by 0.5 days; altered gender balance; etc.
(implication; relevance?)

Ab: hyperactivity (species extrapolation; implication?)

B. Epidemiological studies

Ba: Parkinsonism & childhood leukemia in areas of high pesticide use
(plausibility, causality?)

Bb: Methylmercury from fish intake and cognitive performance
(modulation by nutrients; causality; confounding?)

C. In vitro studies

Ca: Positive outcome in the embryonic stem cell test (EST)
(relevance; association with adverse outcome?)

Cb: Zebra fish altered movement in the dark
(relevance; association with adverse outcome?)

Two reasons to consider mechanisms

II. Generation of data

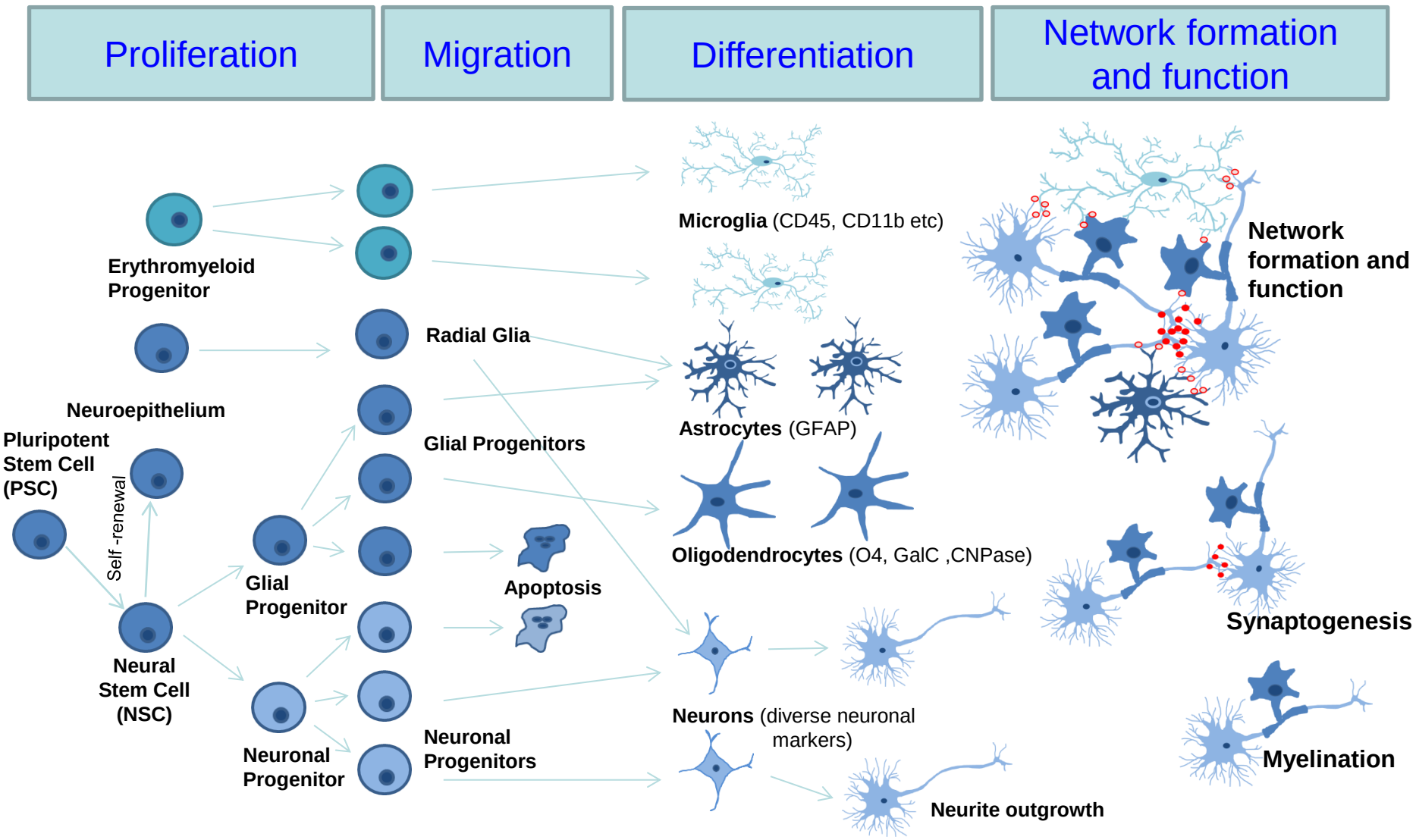
Principle: ‚process control' instead of ‚end stage control'

Assumption I: there are **key neurodevelopmental processes** required to form a fully functional and intact nervous system.

Assumption II: if key neurodevelopmental processes are disturbed, functional or structural deficits may arise.

Procedure: define and establish test methods for key neurodevelopmental processes and evaluate interference by test chemicals

Key neurodevelopmental processes



Bal-Price (2018) ALTEX

Eventually, any DNT finding (man or animal) must be due to a combination of disturbed neurodevelopmental processes

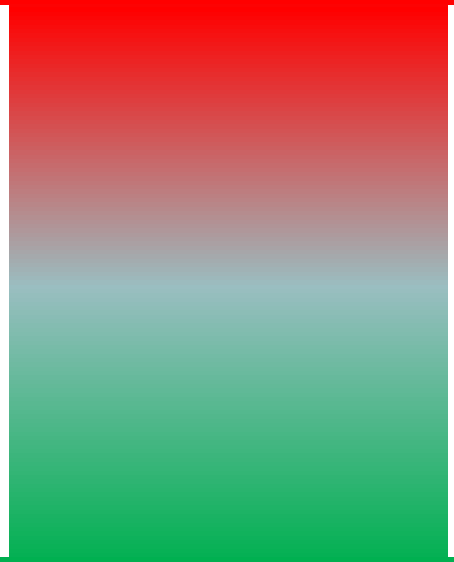
In vivo Finding	Disturbed neurodevelopmental processes
Brain weight up/down	**Proliferation**, **Apoptosis**
Holoprosencephaly	**Apoptosis**, **Neurodifferentiation**
Lissencephaly	**Apoptosis**, **Neurodifferentiation**, **Migration**
Neuroinflammation	**Astrocyte activation, Gliosis**, **Neurodegneration**
Cortical layer thickness	**Proliferation**, **Migration**, **Myelination**
Disturbed reflexes	**Neurodifferentiation**, **Myelination**, **Synaptic transmission**
Anxiety behaviour	**Neurodifferentiation**, **Synaptic transmission, Synapse formation**

If a compound does not disturb at least one process, it cannot be associated with a DNT hazard

Two reasons to consider mechanisms

II. Generation of data: expectations and challenges

De novo (no prior knowledge) evaluation of a new unknown compound for classification and labelling



Screening of libraries of compounds to check for ‚alerts' and to prioritize for further more comprehensive (resource-consuming) testing

alert: preliminary indication that there is a hazard potential; needs verification by other methods

Two reasons to consider mechanisms

II. Generation of data: expectations and challenges

De novo (no prior knowledge) evaluation of a new unknown compound for classification and labelling

Read-across (RAX):
1. anchoring toxicity of unknown compound by comparison to similar* known compound (s);
2. comparison within a category of related* compounds

Screening of libraries of compounds to check for ‚alerts' and to prioritize for further more comprehensive (resource-consuming) testing

**** similarity*** *extended from structure to mechanisms (and metabolism)*

Two reasons to consider mechanisms

I. Making sense of data

Plausibility, relevance
Species extrapolation
Causality

II. Generation of data

Key neurodevelopmental processes
Gap-filling / screening/ prioritization
Read-across (RAX)
De novo evaluation

What is wrong with descriptive data

(Often outdated technology)

Data always describe a **model** – **not the reality**!

→ always an extrapolation required (**uncertainty**)
→ poor explanation of uncertainty
→ implicit mechanistic assumptions (not rationalized and validated)

Example: mouse cancer bioassay
Perfect description, but wrong model (< 60% concordance)

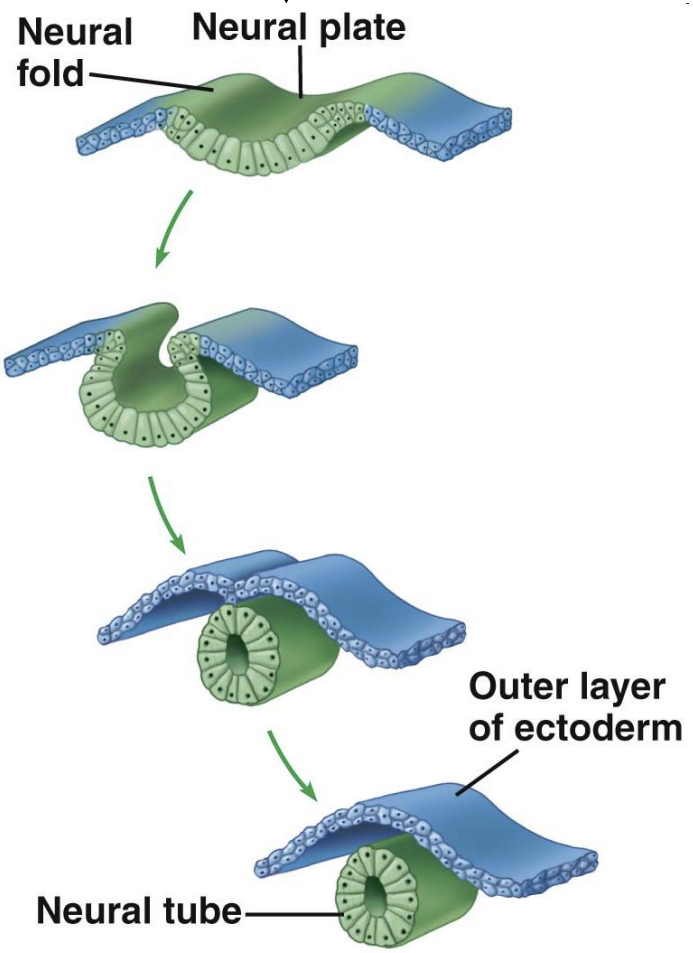
Is a mechanistic approach less direct?

Level	Parameter
Direct observation	Altered light-dark behaviour

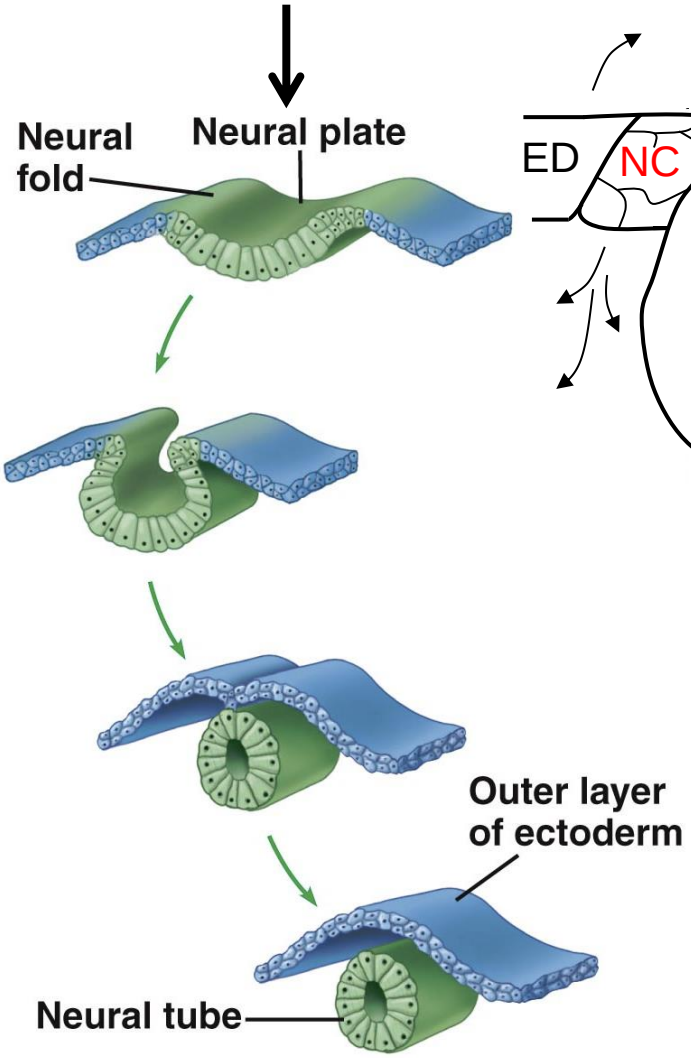
Is a mechanistic approach less direct?

Level	Parameter	Human situation
Direct observation	Altered light-dark behaviour	Meaningless
Interpretation (theoretical construct)	Anxiety	
Endophenotype (measurable change in structure or connectivity)	Altered function/structure of amygdala (limbic system)	
Processes disturbed (during development)	Migration/Differentiation	
Mechanistic correlate / endpoint	Hit in Migration/Differentiation assay	

Example:
Test of early brain/spinal cord development

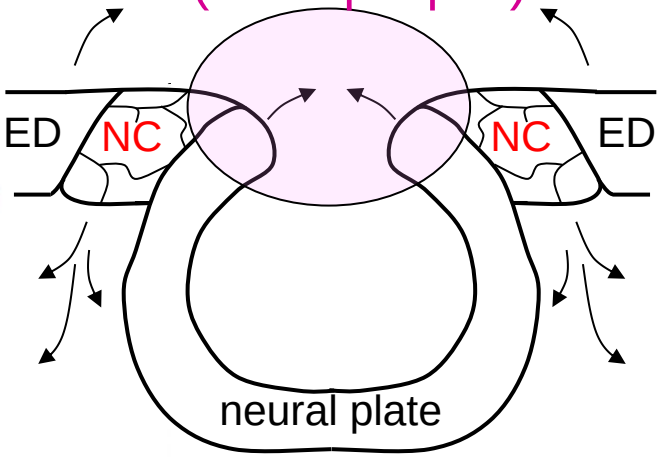


forms spinal cord

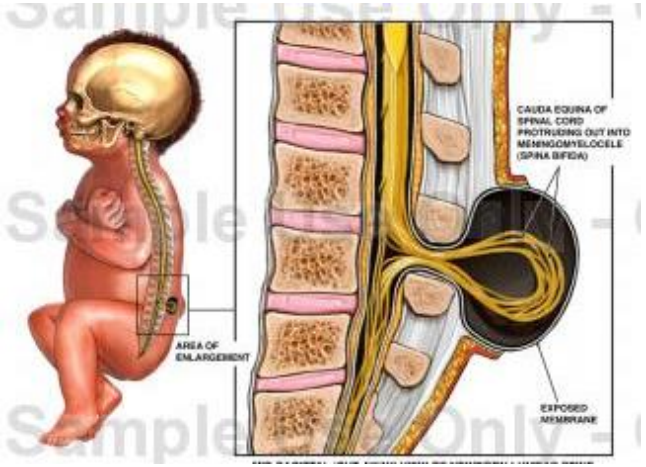


forms spinal cord

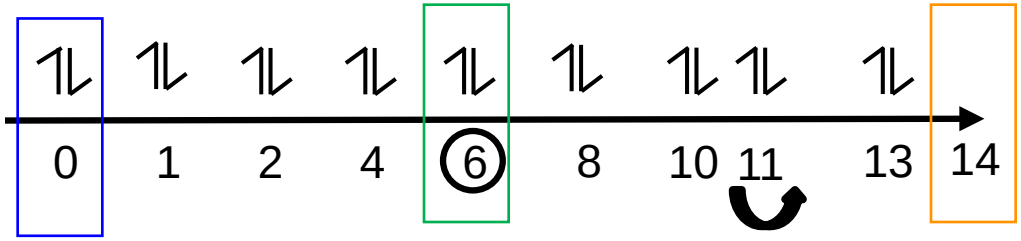
Valproic acid (**VPA**)
(anti-epileptic)

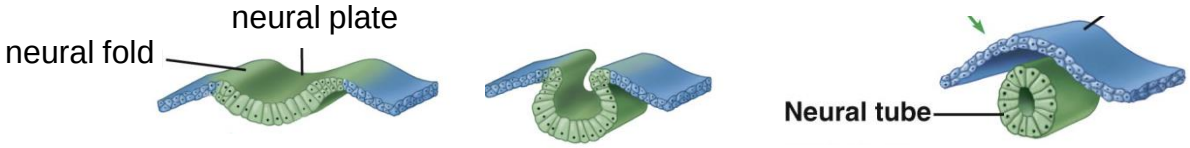


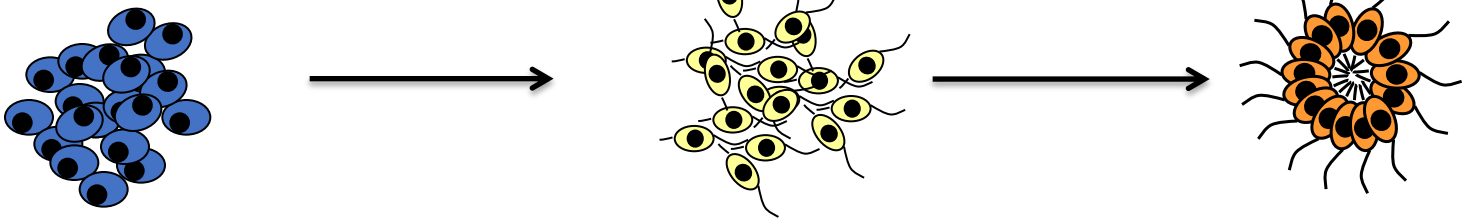
→Failure of neural tube closure



Cellular model: Neural differentiation from hiPSC







iPSC (Oct4, Nanog)

day -3

human pluripotent stem cells

NEP (Pax6, Otx2)

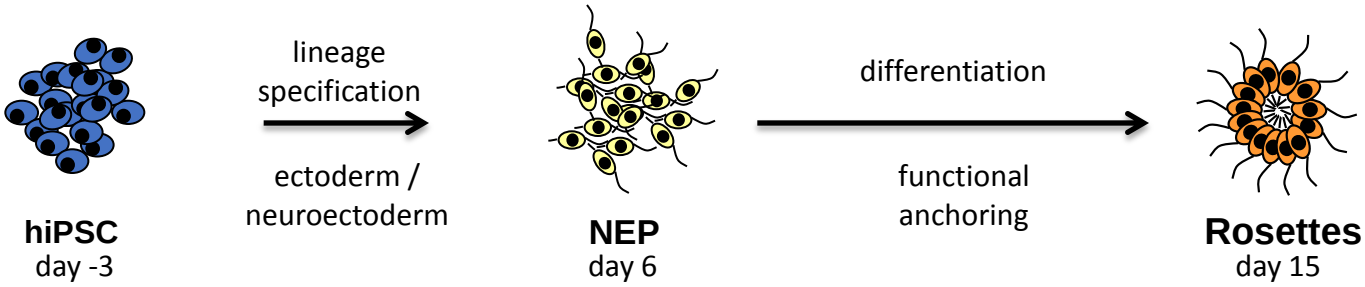
day 6

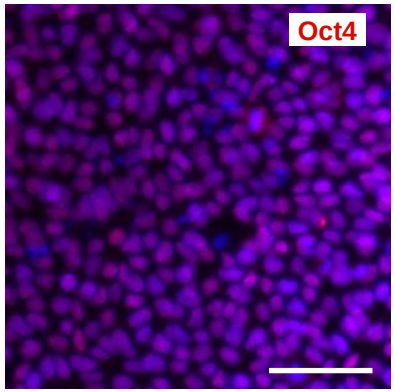
neuroectodermal progenitors

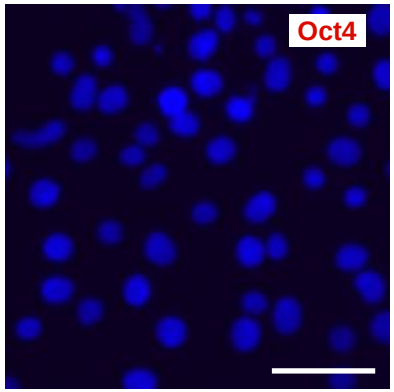
Rosettes

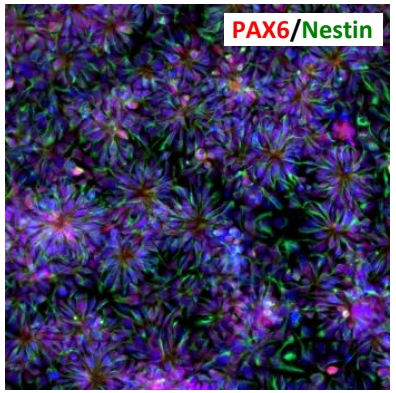
day 14

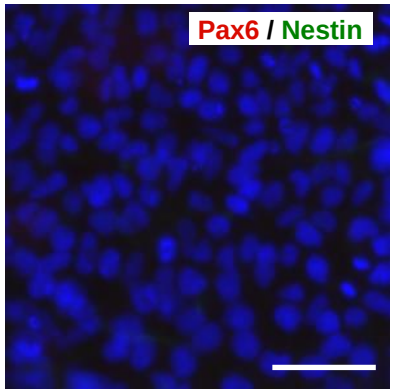
Cellular model: Neural differentiation from iPSC

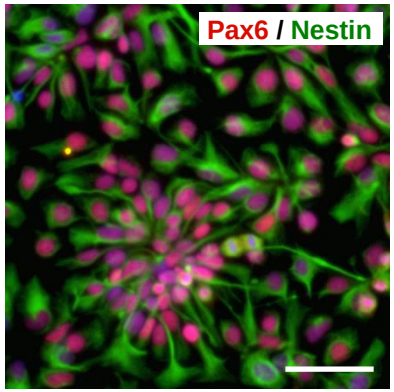


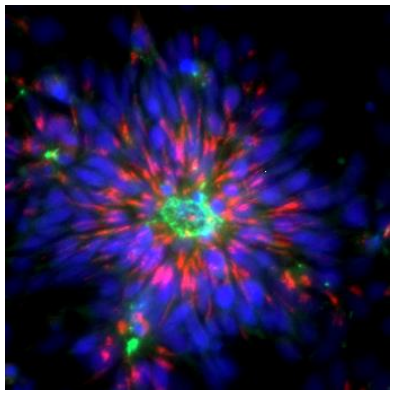




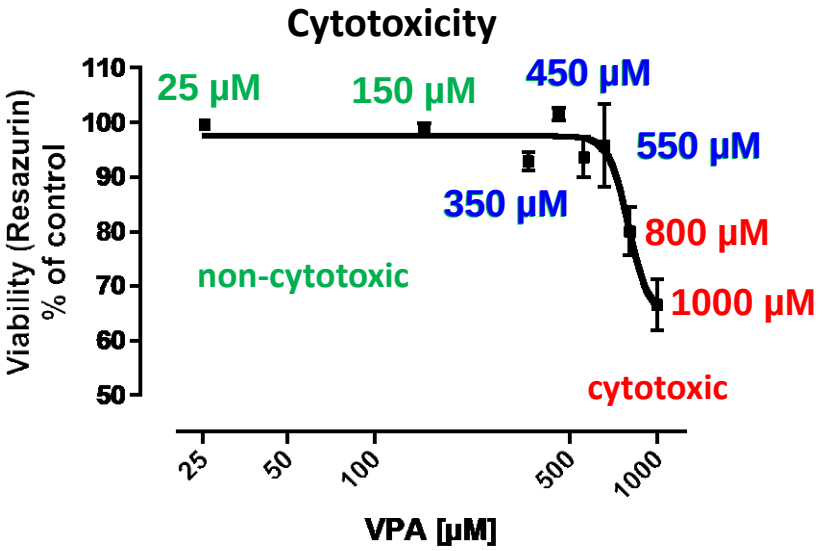






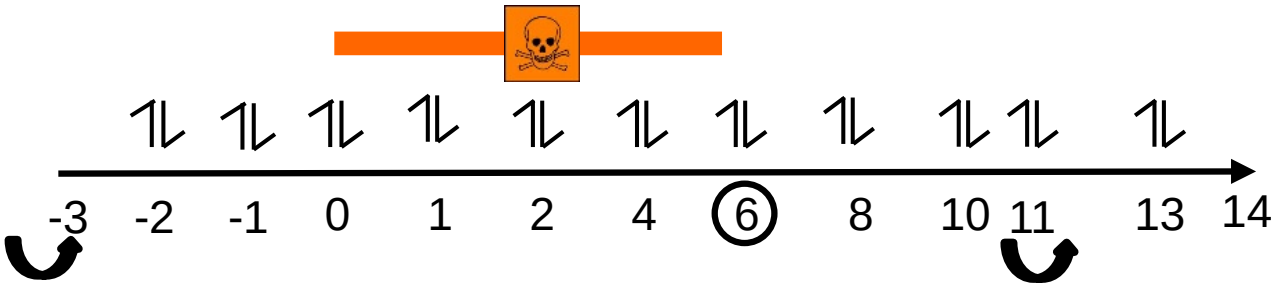


Relevant concentration range – concentration response of valproic acid (VPA)

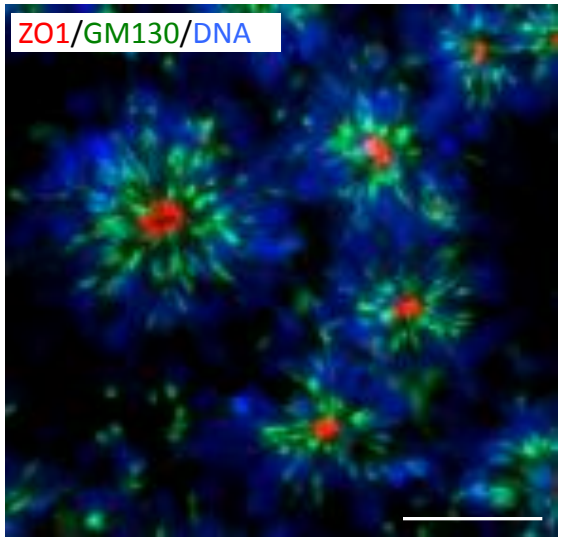


➔ Gene expression changes start with 350 µM VPA
➔ no cytotoxicity observed in this range

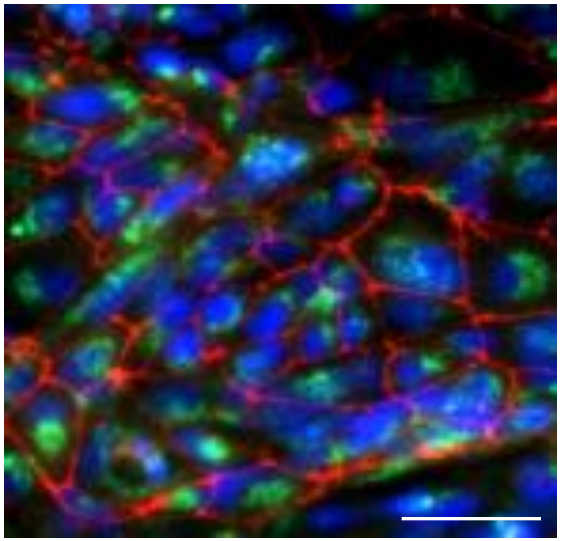
Functional anchoring after VPA treatment

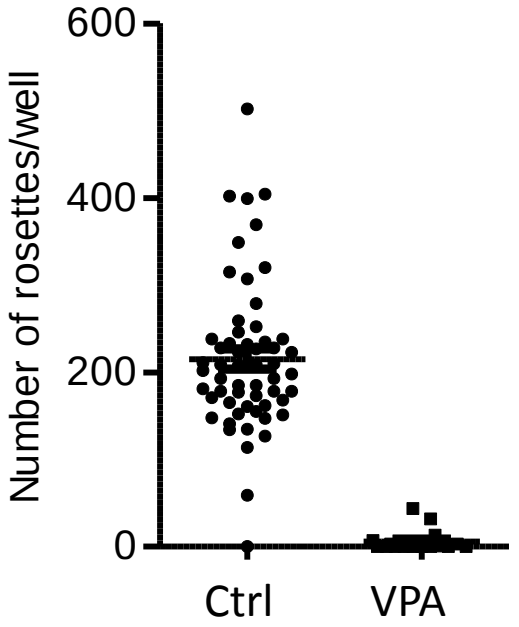


Control (solvent only)

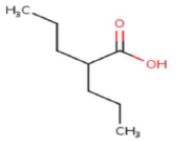
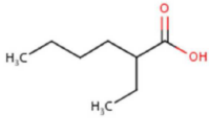
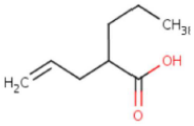
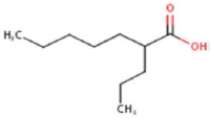
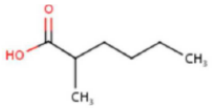
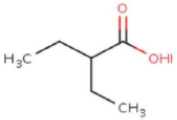
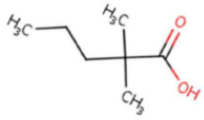
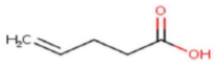
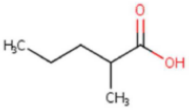
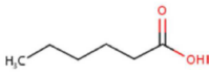


VPA DoD 0-6 (0.6 mM)





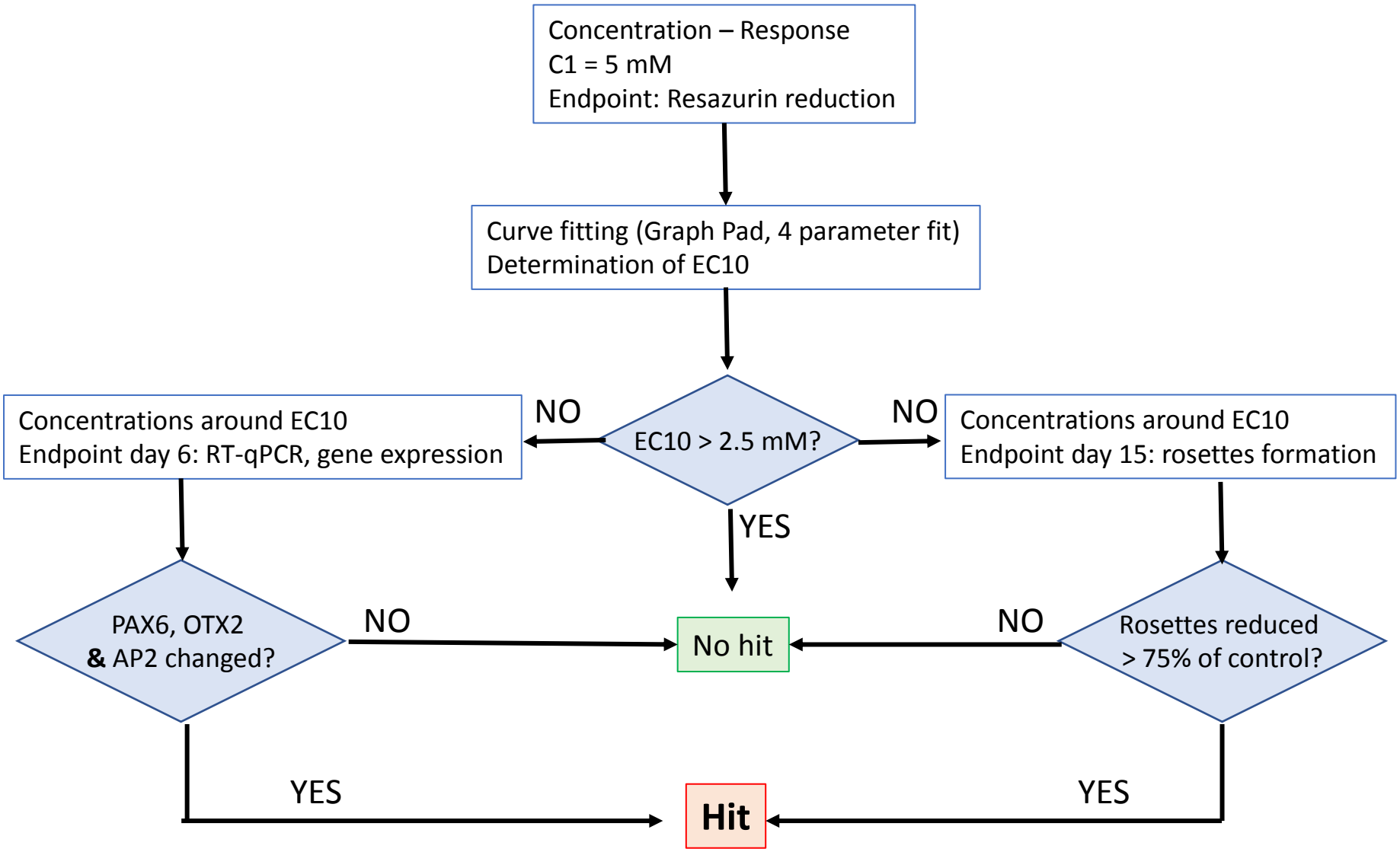
➔ After 6 days of treatment rosettes formation is disturbed
➔ The gene expression changes have functional consequences on differentiation

VPA analogues	CAS-Nrs	structure	In vivo response
VPA	99-66-1		+++
2-ethyl-hexanoic acid	149-57-5		+++
4-ene-VPA	1575-72-0		++
2-n-propyl-heptanoic acid	31080-39-4		+++
2-methyl-hexanoic acid	4536-23-6		-
2-ethyl-butyric acid	88-09-5		-
2,2-dimethyl-pentanoic acid	1185-39-3		-
Pentenoic acid	109-52-4		-
2-methyl-pentanoic acid	97-61-0		?
Hexanoic acid	142-62-1		?

Valproate (VPA) analogues and their *in vivo* response

EUTOXRISK

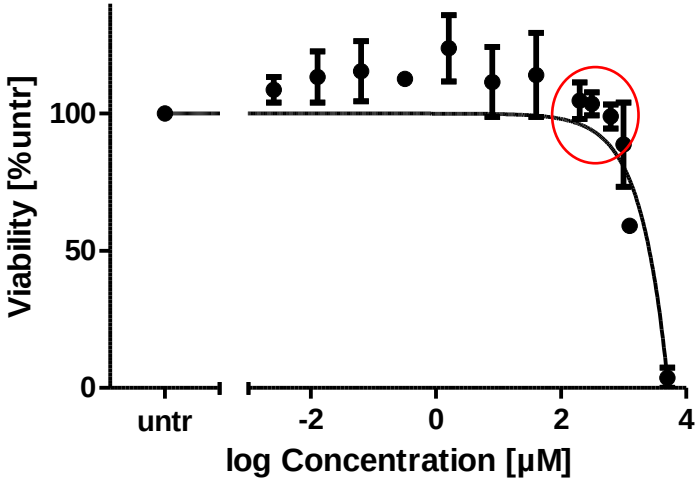
Testing Strategy

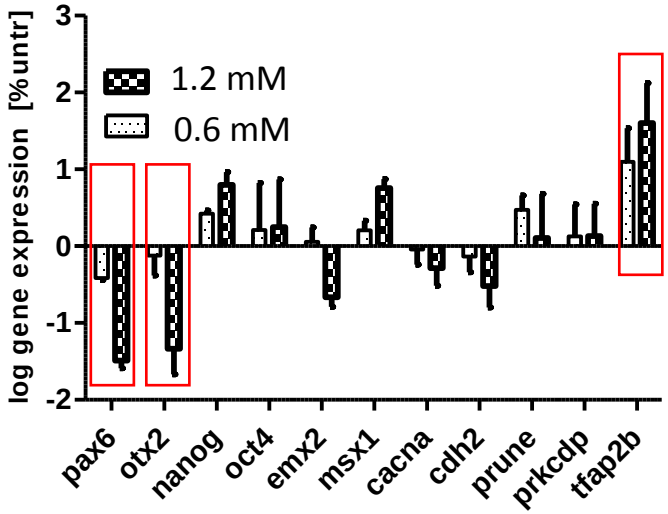


Example for a hit: 4-ene-VPA

Gene expression: 1.2 & 0.625 mM

Viability





➔ Testing in non-cytotoxic range
➔ Expected gene expression changes
➔ Inhibition of rosettes formation

Summary Table

3 clear hits:

➔ Valproic acid	in vivo positive ✓
➔ 2-Ethylhexanoic acid	in vivo positive ✓
➔ 4 ene VPA	in vivo positive ✓

3 are unclear:

➔ Hexanoic acid	in vivo unknown
➔ 2-Methylhexanoic acid	in vivo negative
➔ 2-methyl-pentanoic acid	in vivo unknown

2 clear Negatives:

➔ 2 Ethylbutyric acid	in vivo negative ✓
➔ 2,2-Dimethylvaleric acid	in vivo negative ✓

Results from a test battery

Analogues	In vivo NTD	ZET EC10	ZET reporter	EST (c) IC50	UKN IC10	CALUX
VPA	+++	10	+++	378	600*	
4-ene-VPA	++		++	518	534*	
2-ethyl hexanoic acid	+++		++	1115	943*	
2-propyl heptanoic acid	+++	10	+++	365	208*	
2-ethyl butyric acid	-		-	>3000		
2,2-dimethyl pentanoic acid	-		+	>3000		
2-methyl pentanoic acid	?	250	+	>3000		

(note: data without PBPK correction)

negative (in vitro / in vivo)

unclear/ intermediate (in vitro / in vivo)

positive (in vitro / in vivo)

EUTOXRISK

Summary

1. Mechanistic risk assessment adds value to data

2. Mechanistic risk assessment allows for new NAM-based approaches

3. A battery of tests for key neurodevelopmental processes is available and has been successfully used in case studies

4. There is an educational need on all sides to understand strengths and weaknesses of the new approaches; discussions of case studies can provide a platform

Acknowledgement

Thomas Hartung

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EUTOXRISK

Bob van de Water
Hennicke Kamp and many others