EUTOXRISK

CAAT eu

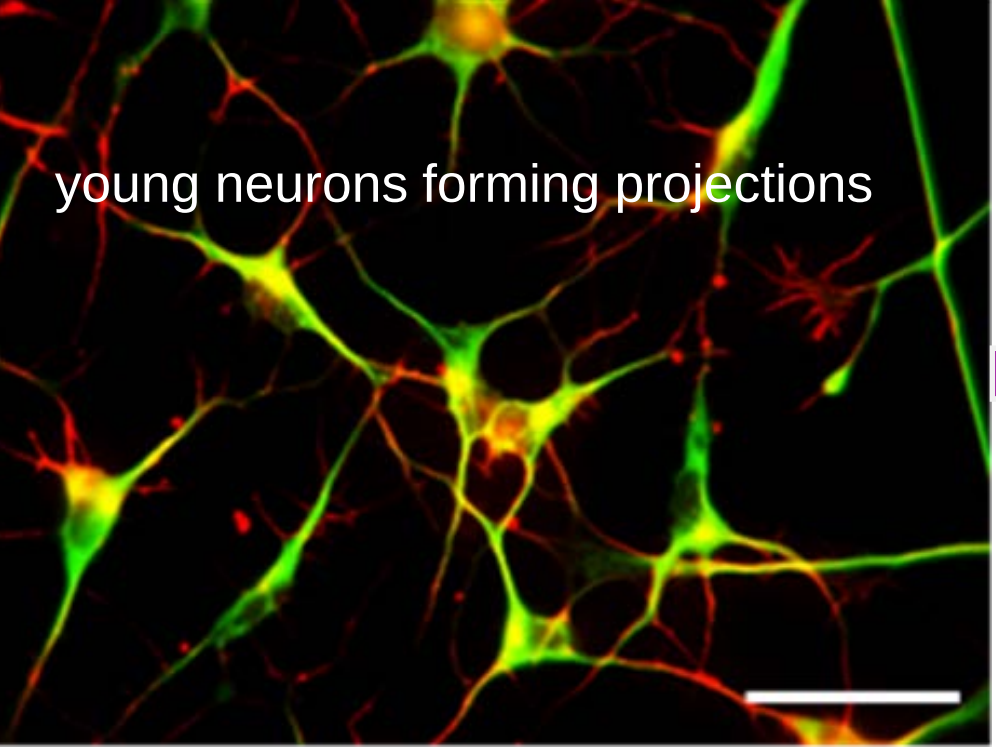
Universität Konstanz

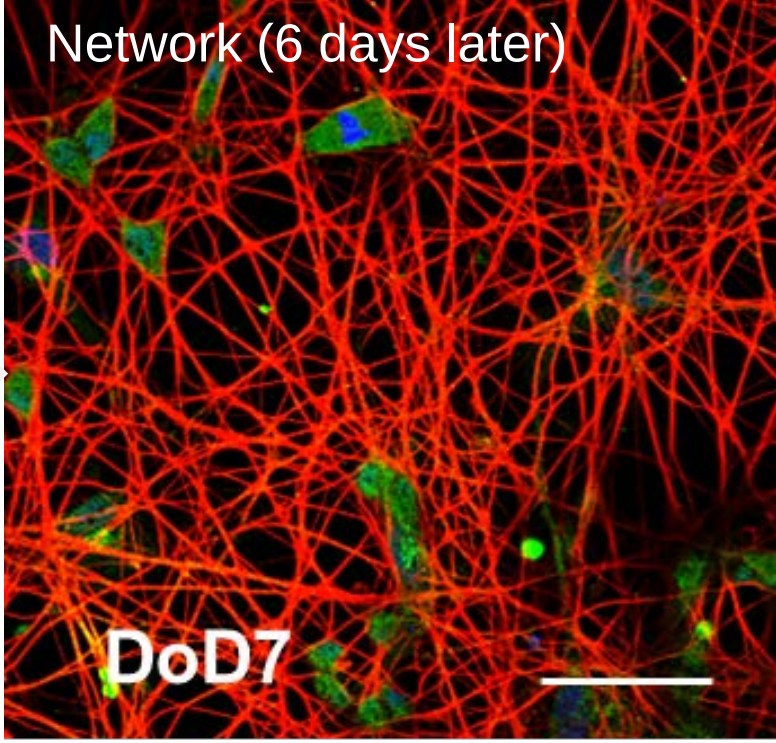
JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

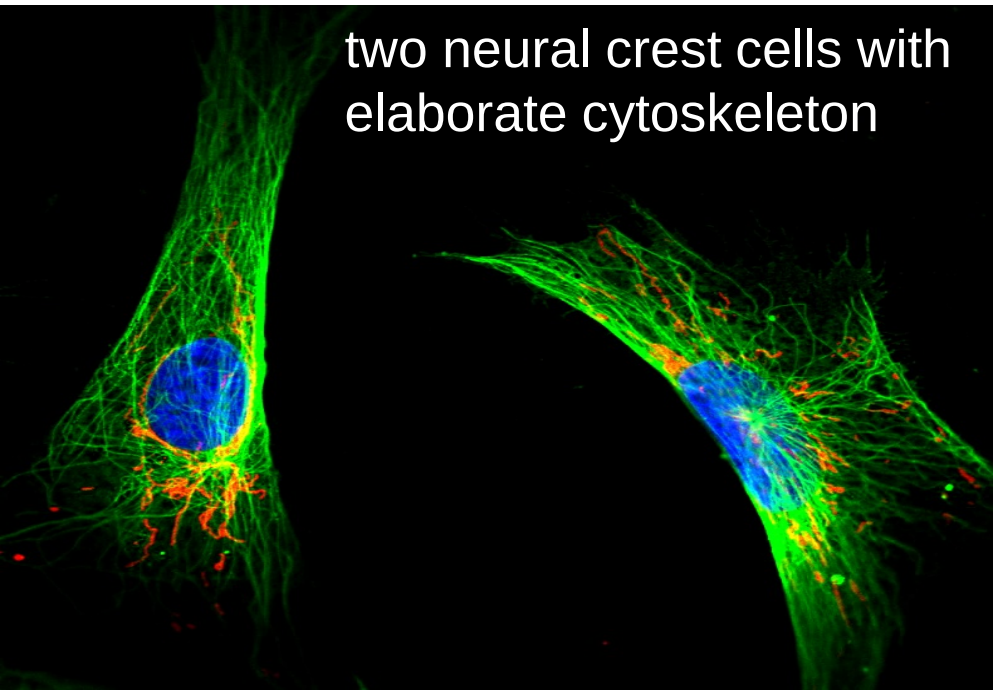
10th Berlin Workshop on Developmental Toxicology

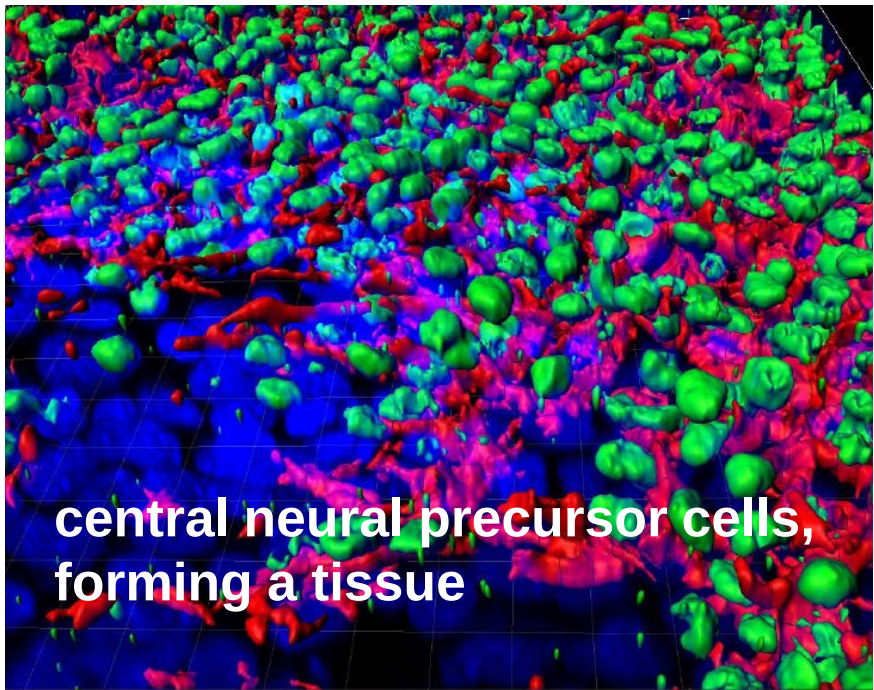
Linking test systems to the prediction of DNT

Marcel Leist
Professor for In Vitro Toxicology and Biomedicine
Chair inaugurated by the Doerenkamp-Zbinden Foundation,
University of Konstanz, Germany

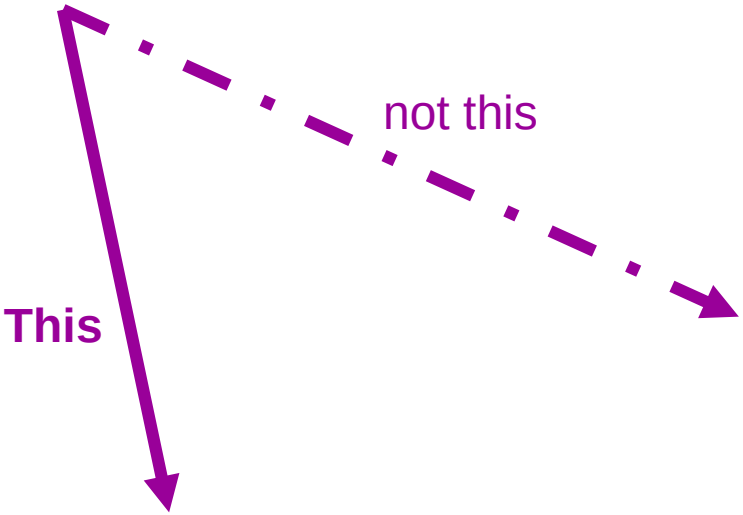

young neurons forming projections

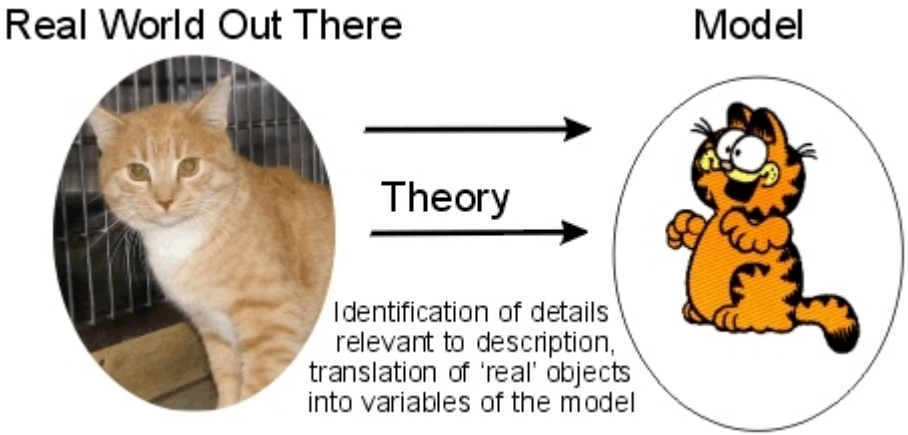

Network (6 days later)
DoD7


two neural crest cells with
elaborate cytoskeleton

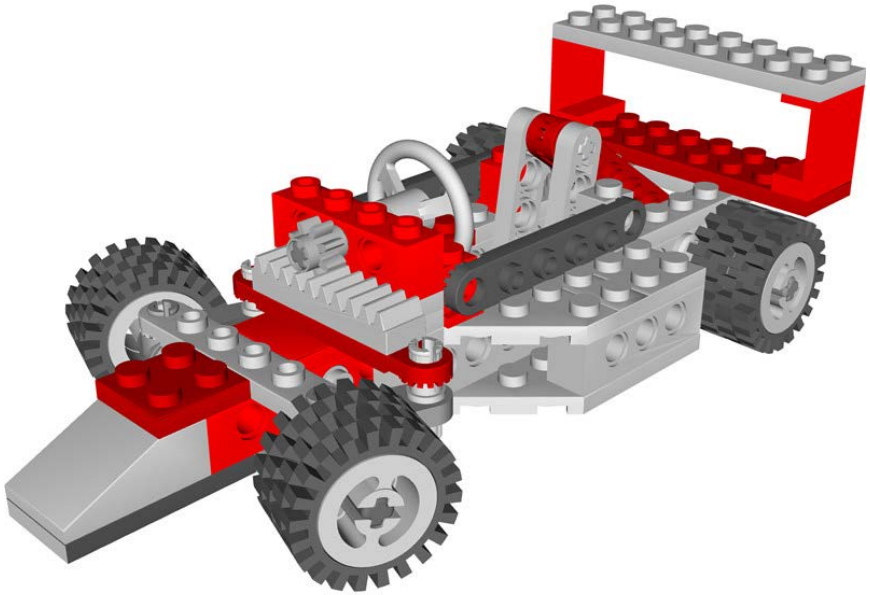

central neural precursor cells,
forming a tissue

A bit of model theory……





Type 1 (replica models): ‚look similar‘



a ‚car‘

Advantages: no need to know, how car functions

Disadvantages: may give wrong answers (e.g. concerning car crashes)

Assumption: if it looks like reality, it will behave like reality under challenge

Type 2 (concept models): ‚function similarly‘

a ‚crash test dummy‘

Advantages:	measurement and prediction of complex events

Disadvantages:	1. does not look like reality 2. need to know how model and reality function 3. works only for very specific questions

Assumption: it functions like reality,

Different approaches to toxicity testing

Type 1 models
(,look‘ similarly**)**

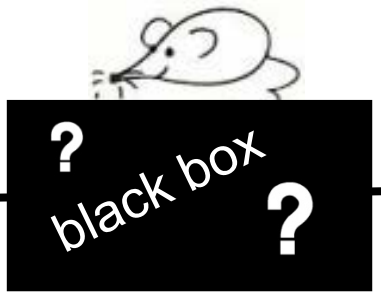
chemicals

→ hazard?

Type 2 models
(function similarly**)**

Different approaches to toxicity testing

Type 1 models
(‚look' similarly**)**



Input→Output relation for animal = man

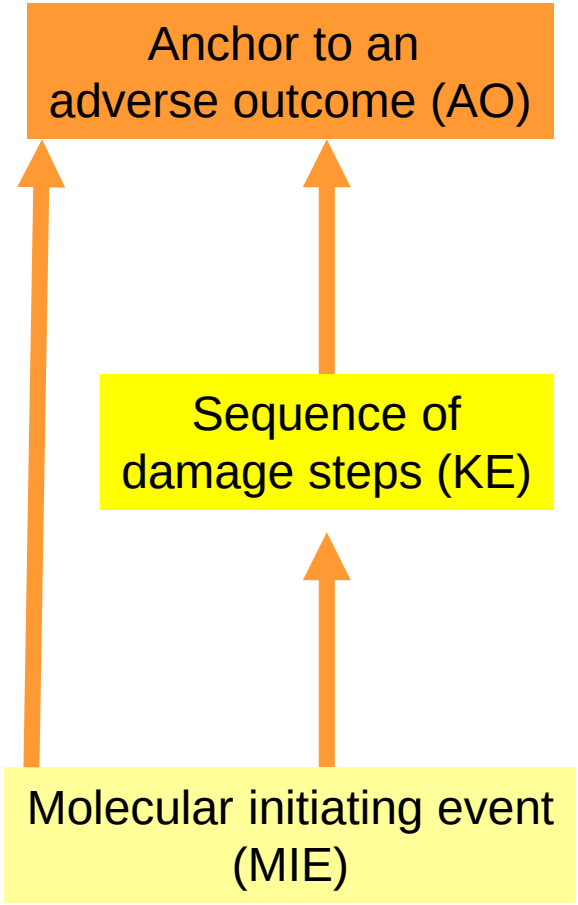
„Early" animal-free tests
- skin irritation
- eye irritation
- genotoxicity
- etc.

Type 2 models
(function similarly**)**

Complex animal-free tests
- non-genotoxic cancer
- **developmental toxicity**
- organ toxicity
- etc.

Approaches to use functional testing for hazard assessment

(= use of new approach methods (NAM); mechanistic assays)



Arch Toxicol (2017) 91:3477–3505
DOI 10.1007/s00204-017-2045-3

REGULATORY TOXICOLOGY

Adverse outcome pathways: opportunities, limitations and open questions

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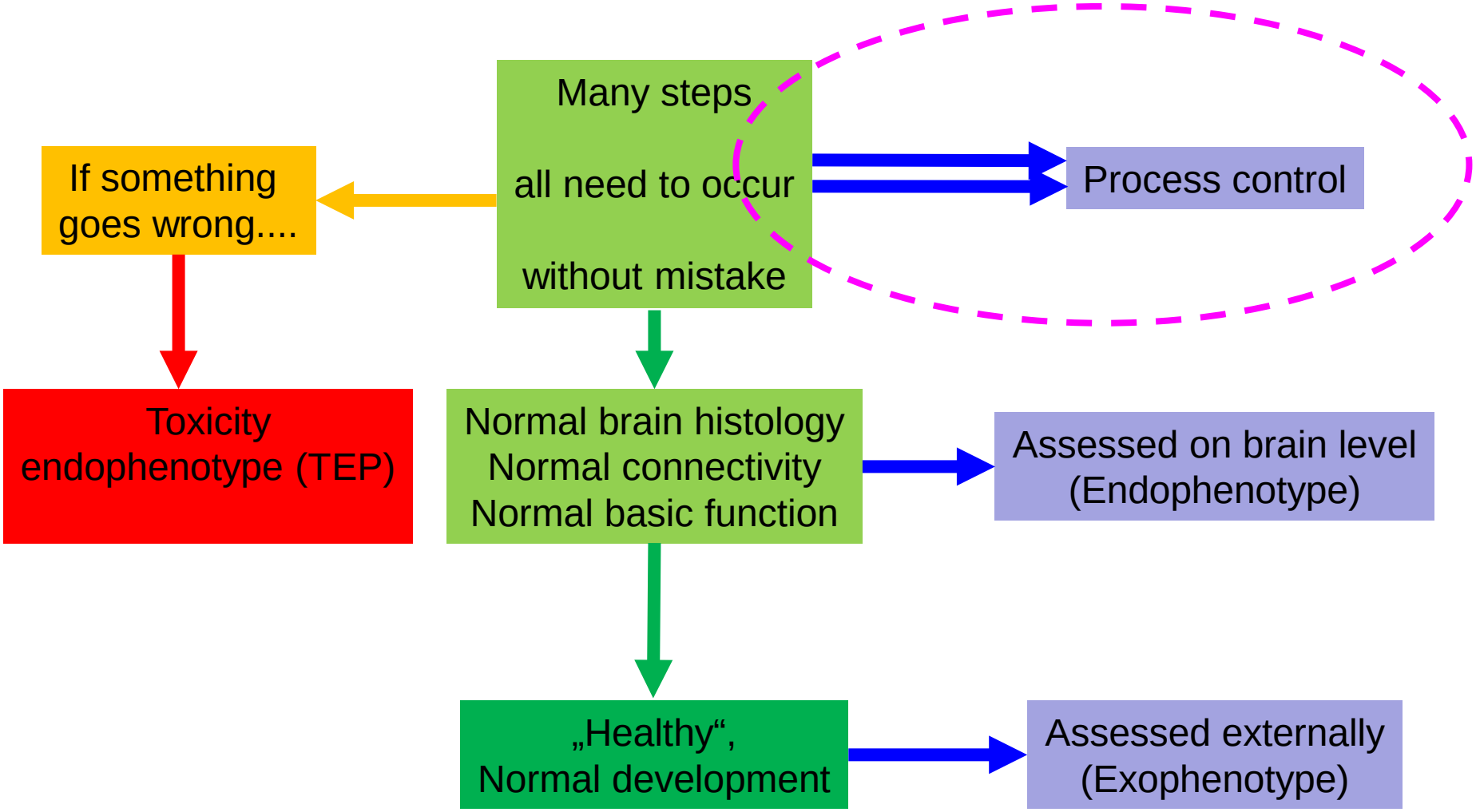
Damage assessment, but at earlier stages

Alternative: non-damage assessment, „all going well“

Approaches to use functional testing for hazard assessment

(= use of new approach methods (NAM); mechanistic assays)



Principle:

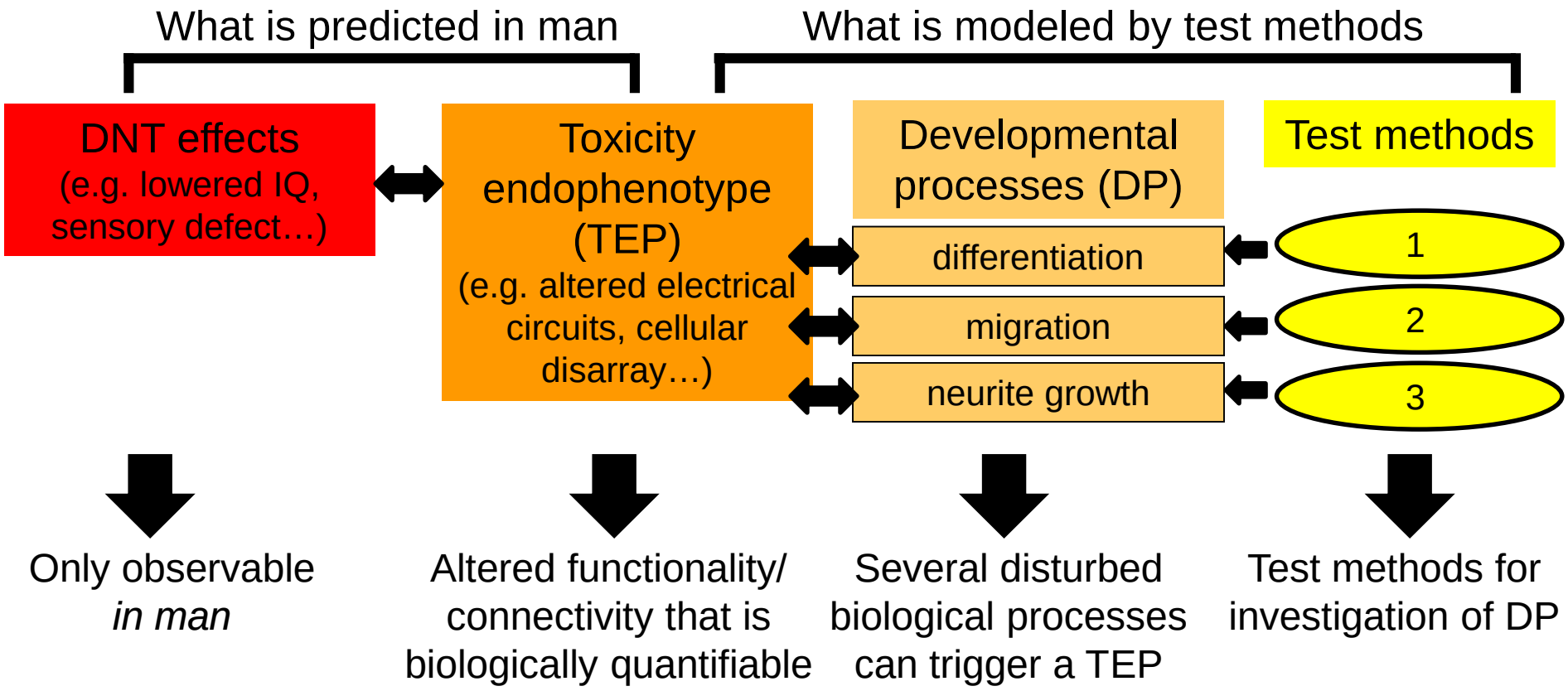
‚process control‘ instead of ‚end stage control‘

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

Overview: a process control-based test strategy for DNT

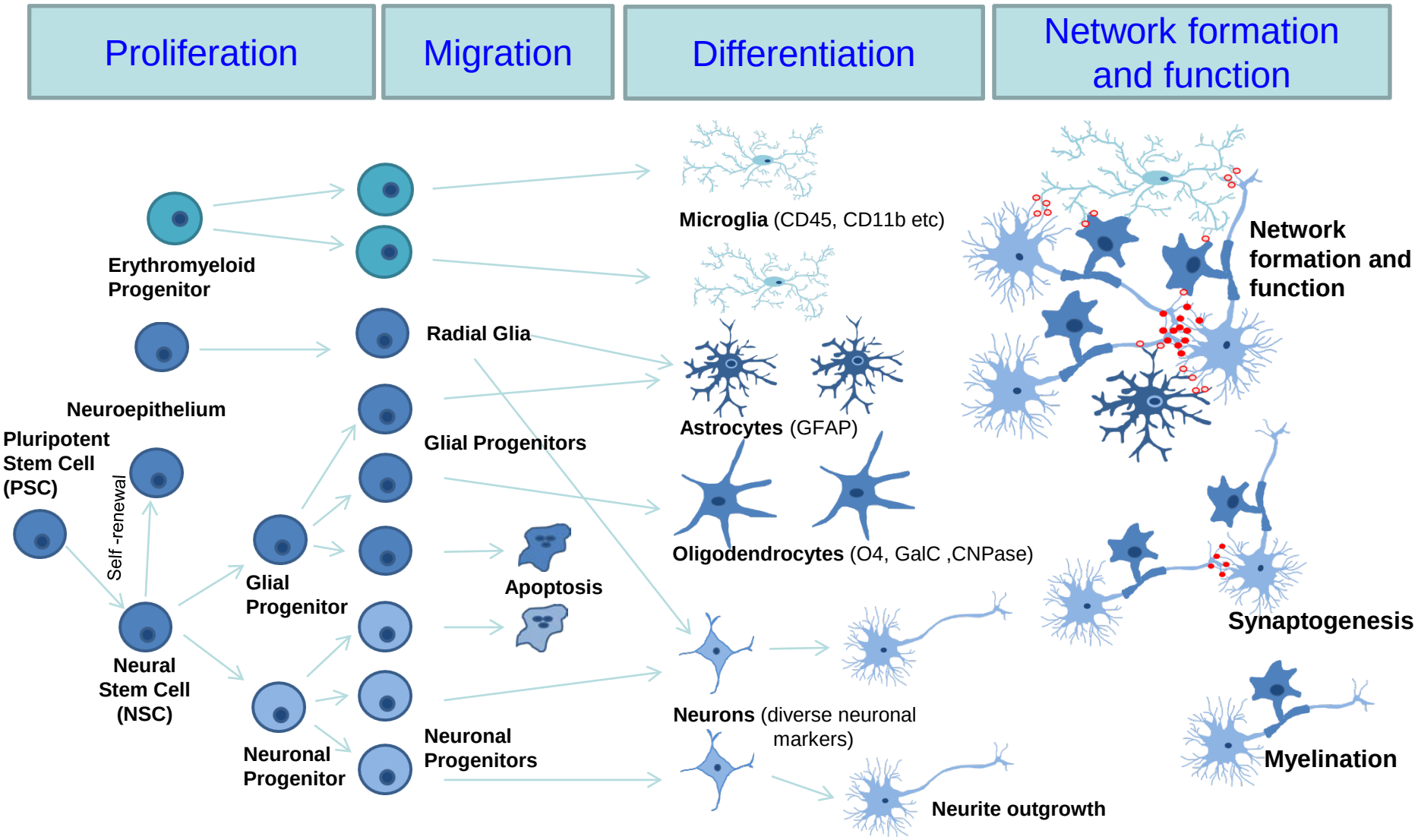


DP = fundamental neurodevelopmental processes in DNT

Addressing the underlying endophenotypes is feasible

this is all nice, but the cells (2D / 3D/ …) neither think nor feel nor talk nor sleep nor see

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

Ongoing projects to cover neurodevelopmental processes by NAM

(examples)

1. NTP screen battery (international contributions and data base)

OXFORD

SOT | Society of Toxicology

www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 167(1), 2019, 6–14

doi: 10.1093/toxsci/kfy278

Advance Access Publication Date: November 28, 2018

Forum

FORUM

Screening for Developmental Neurotoxicity at the National Toxicology Program: The Future Is Here

Mamta Behl,*,[1] Kristen Ryan,* Jui-Hua Hsieh,† Frederick Parham,* Andrew J. Shapiro,* Bradley J. Collins,* Nisha S. Sipes,* Linda S. Birnbaum,* John R. Bucher,* Paul M. D. Foster,* Nigel J. Walker,* Richard S. Paules,* and Raymond R. Tice‡

*Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research

Ongoing projects to cover neurodevelopmental processes by NAM
(examples)

2. OECD-coordinated program (with EPA, EFSA, JRC etc..)

OXFORD
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www.toxsci.oxfordjournals.org

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doi: 10.1093/toxsci/kfy211
Advance Access Publication Date: November 23, 2018
Forum

FORUM

International Regulatory and Scientific Effort for Improved Developmental Neurotoxicity Testing

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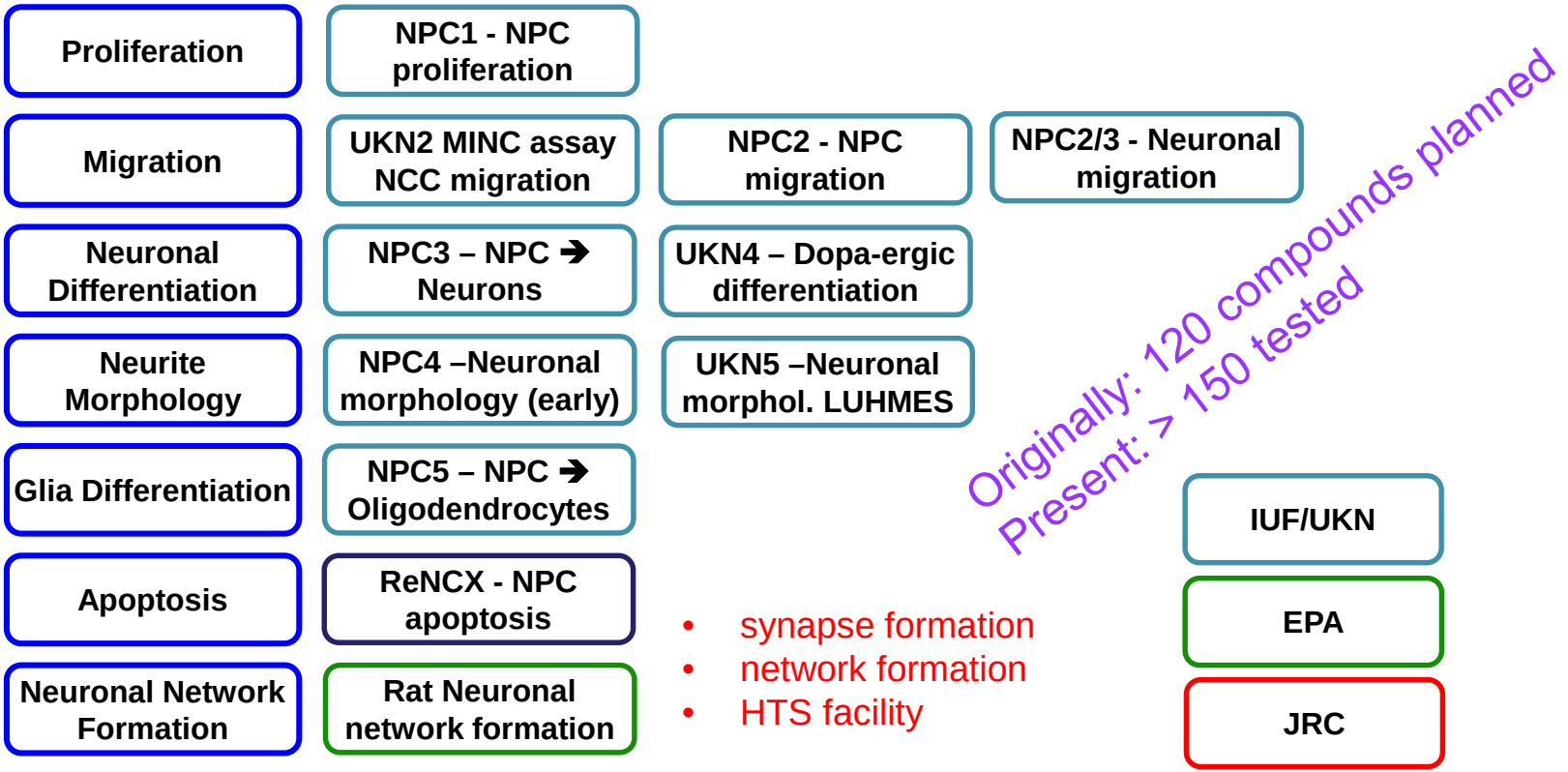
Ongoing projects to cover neurodevelopmental processes by NAM
(examples)

2. OECD-coordinated program (with EPA, EFSA, JRC etc..)

EFSA / DK-EPA screen battery (at IUF and UKN)



IUF: Leibnitz Institute Düsseldorf, UKN: University Konstanz,
EPA: US environmental protection agency, JRC: EURL-ECVAM laboratories

Ongoing projects to cover neurodevelopmental processes by NAM

(examples)

2. OECD-coordinated program (with EPA, EFSA, JRC etc..)

EFSA / DK-EPA screen battery (at IUF and UKN)

test compounds

assays

Heatmap

IUF: Leibnitz Institute Düsseldorf, UKN: University Konstanz,
EPA: US environmental protection agency, JRC: EURL-ECVAM laboratories

Ongoing projects to cover neurodevelopmental processes by NAM
(examples)

3. SysDT project of the BMBF

Archives of Toxicology (2020) 94:151–171
https://doi.org/10.1007/s00204-019-02612-5

IN VITRO SYSTEMS

Development of a neural rosette formation assay (RoFA) to identify neurodevelopmental toxicants and to characterize their transcriptome disturbances

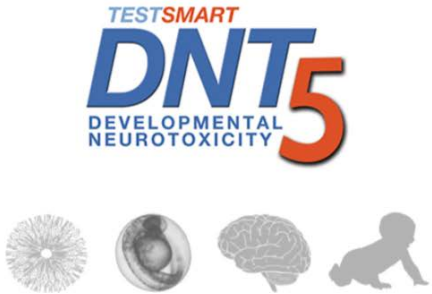
Nadine Dreser[1] · Katrin Madjar[2] · Anna-Katharina Holzer[1] · Marion Kapitza[1] · Christopher Scholz[1] · Petra Kranaster[1,7] · Simon Gutbier[1,8] · Stefanie Klima[1] · David Kolb[3,9] · Christian Dietz[3,9] · Timo Trefzer[1,10] · Johannes Meisig[4] · Christoph van Thriel[5] · Margit Henry[6] · Michael R. Berthold[3] · Nils Blüthgen[4] · Agapios Sachinidis[6] · Jörg Rahnenführer[2] · Jan G. Hengstler[5] · Tanja Waldmann[1] · Marcel Leist[1]

Universities of Konstanz, Köln, Dortmund
Charité Berlin, IfADO Dortmund
associated: Roche (Basel)

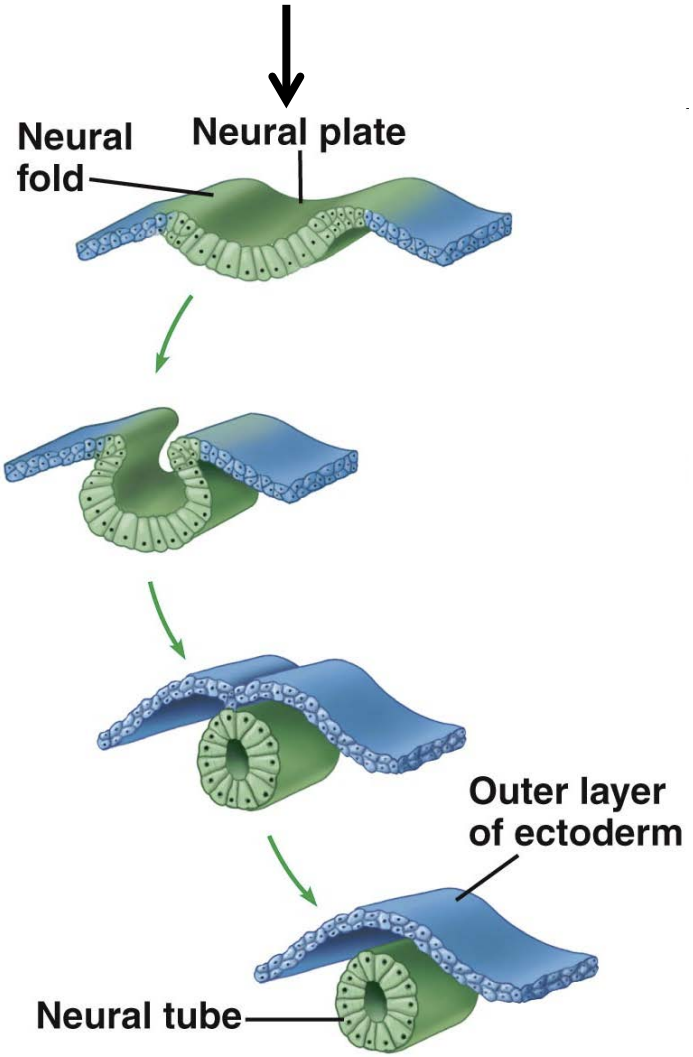
BMBF: German ministry of science and technology

Ongoing projects to cover neurodevelopmental processes by NAM
(examples)

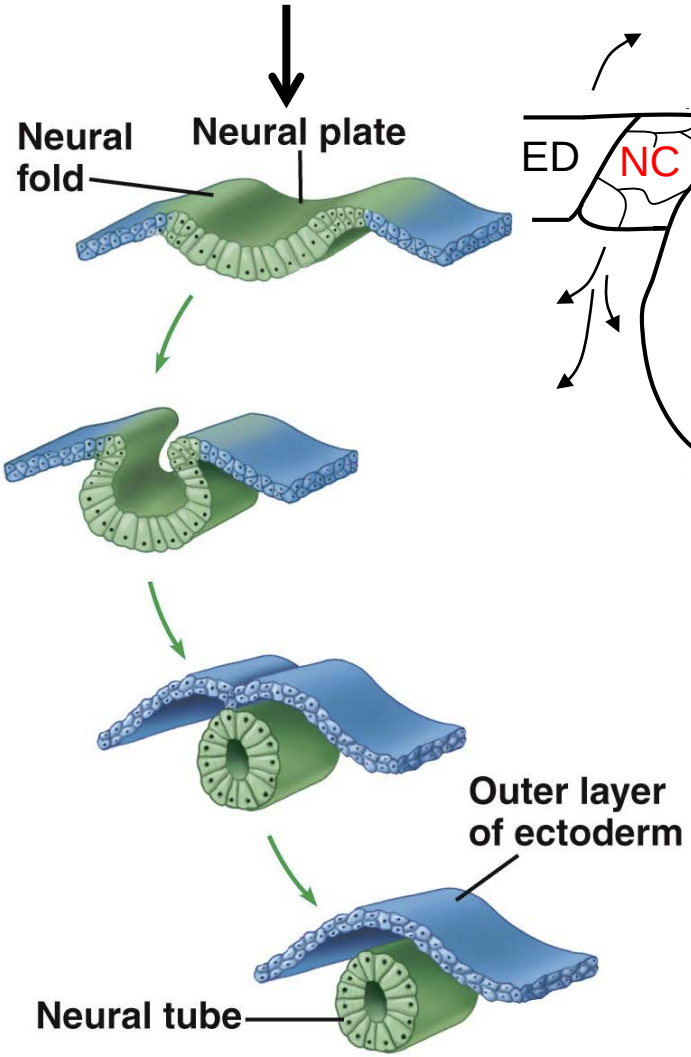
4. DNT meeting series and CAAT workshops



Example:
Test of early brain/spinal cord development

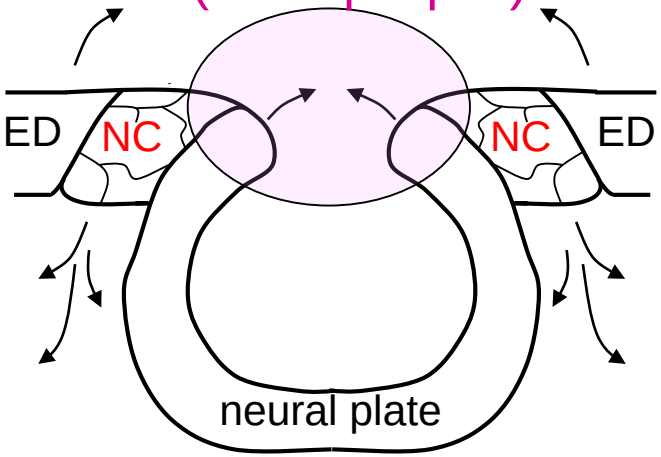


forms spinal cord

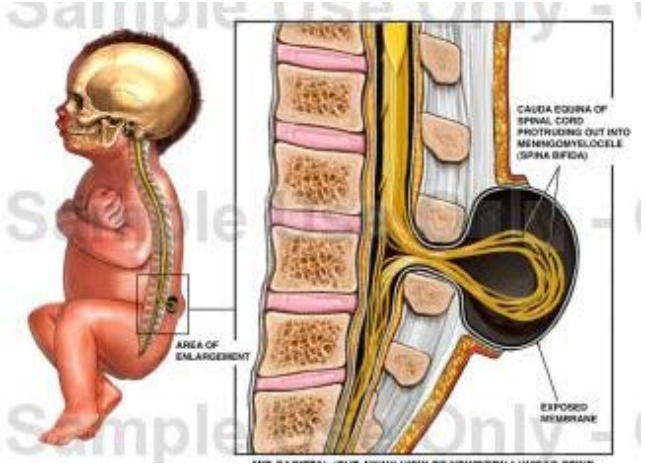


forms spinal cord

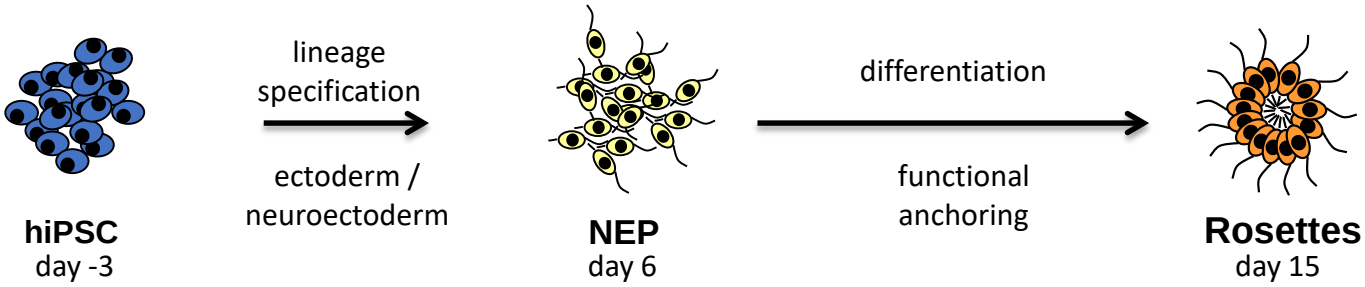
Valproic acid (**VPA**)
(anti-epileptic)

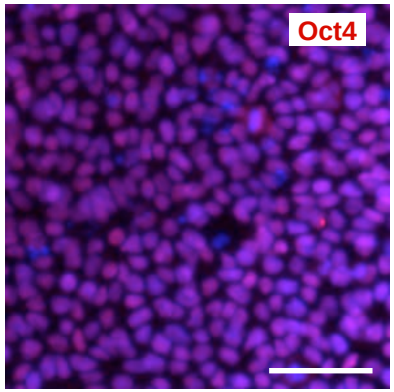


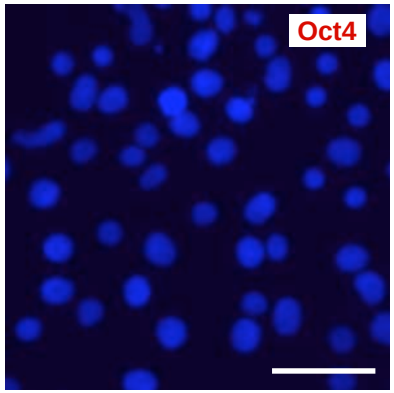
→Failure of neural tube closure

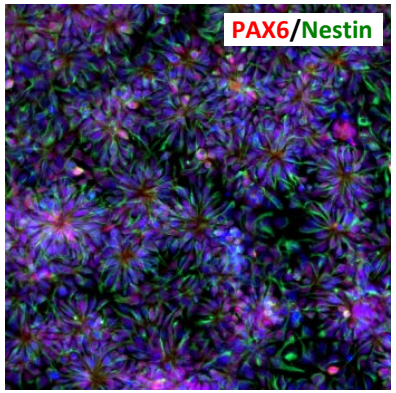


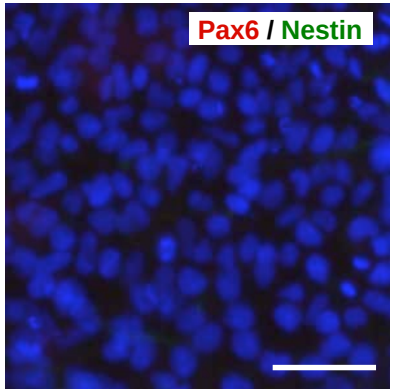
Cellular model: Neural differentiation from iPSC

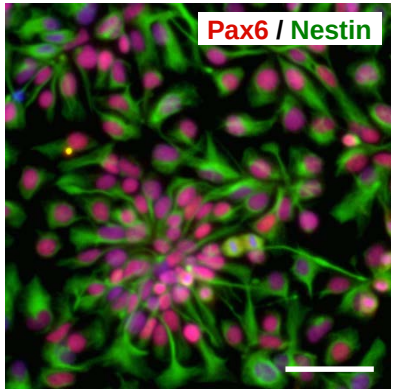


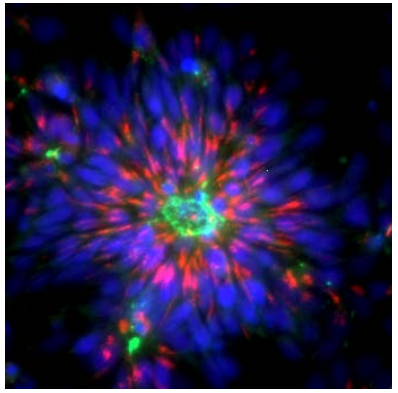








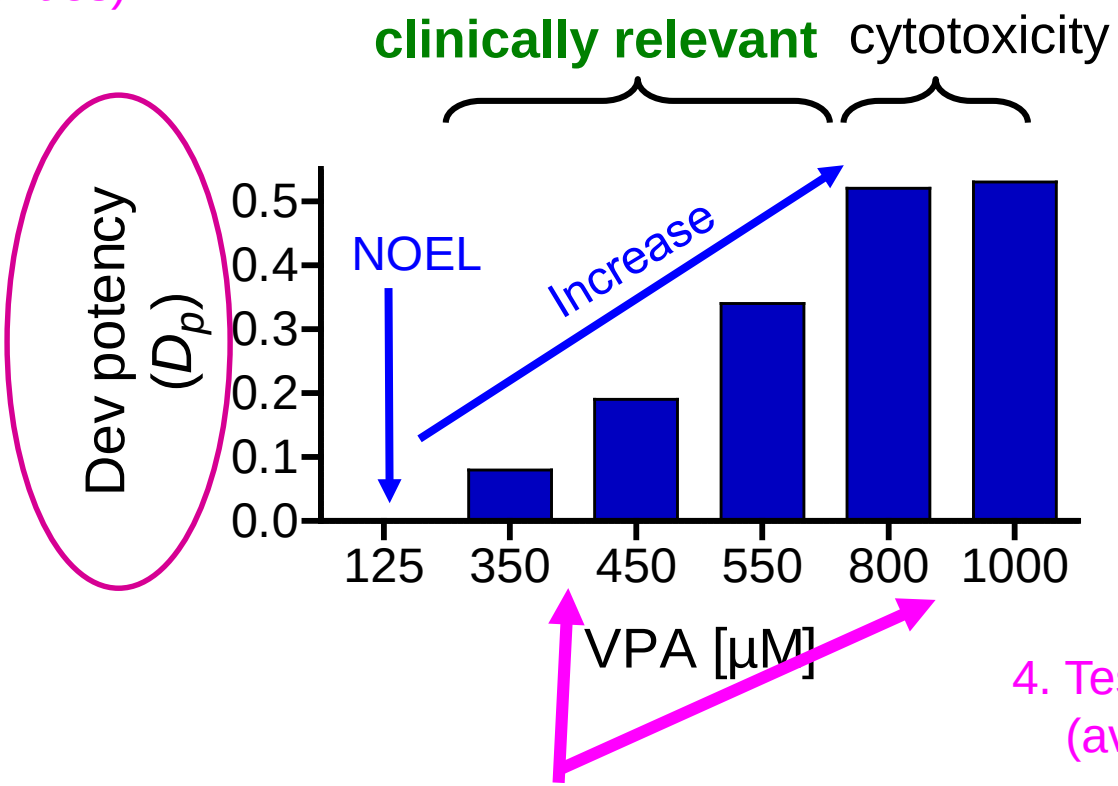




Model readouts and examples for current challenges

1. Robust quantification
(with uncertainties)

3. In vitro – in vivo extrapolation
(with barriers of placenta and brain)



4. Test battery
(avoid FN and FP)

2. Toxicological prediction model
(with performance parameters)

Shinde et al (2016) Arch Toxicol

Acknowledgement