Testing for key neurodevelopmental processes

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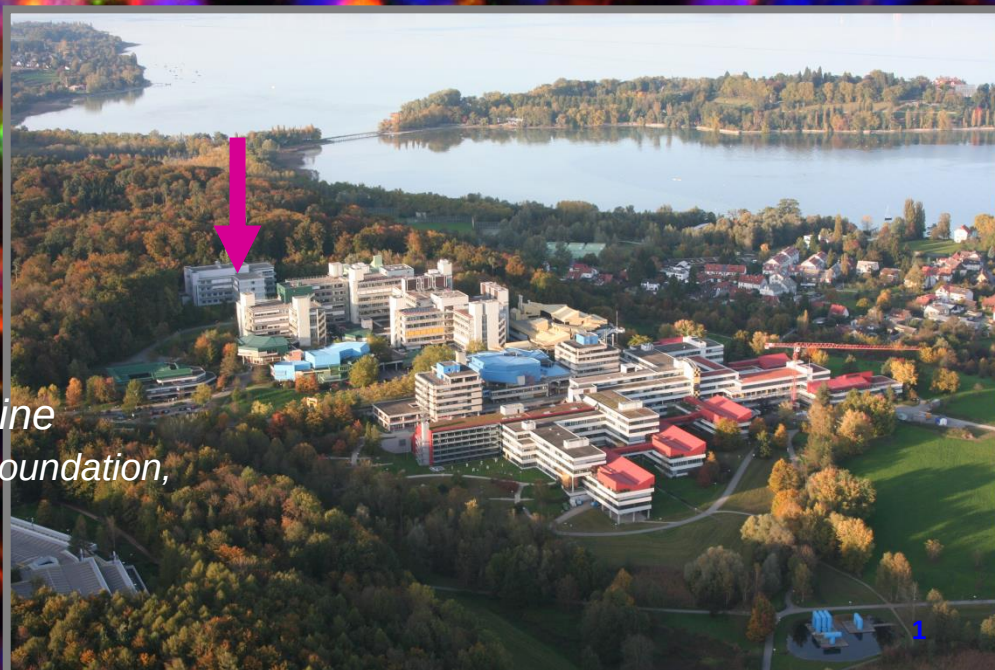
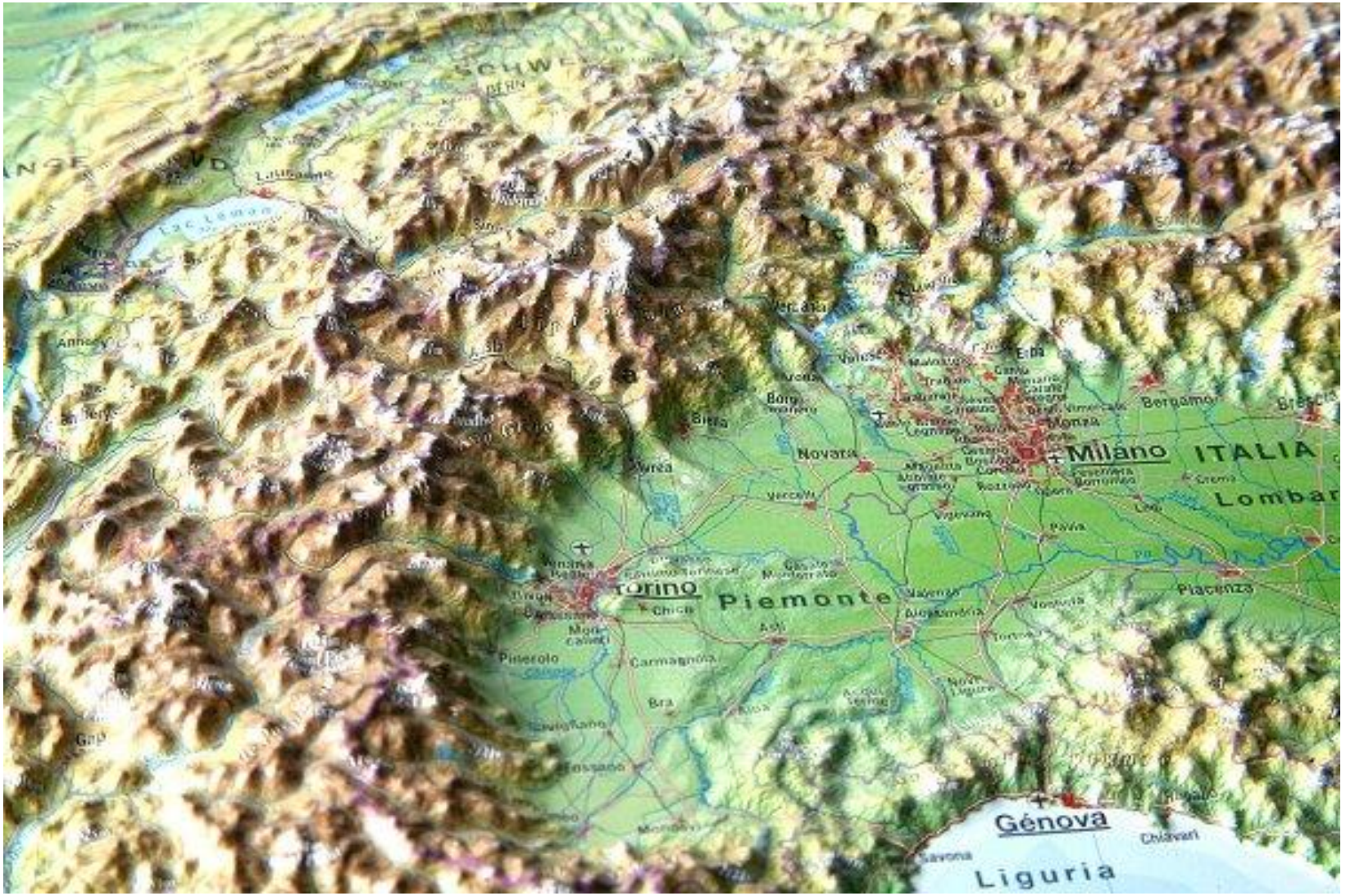
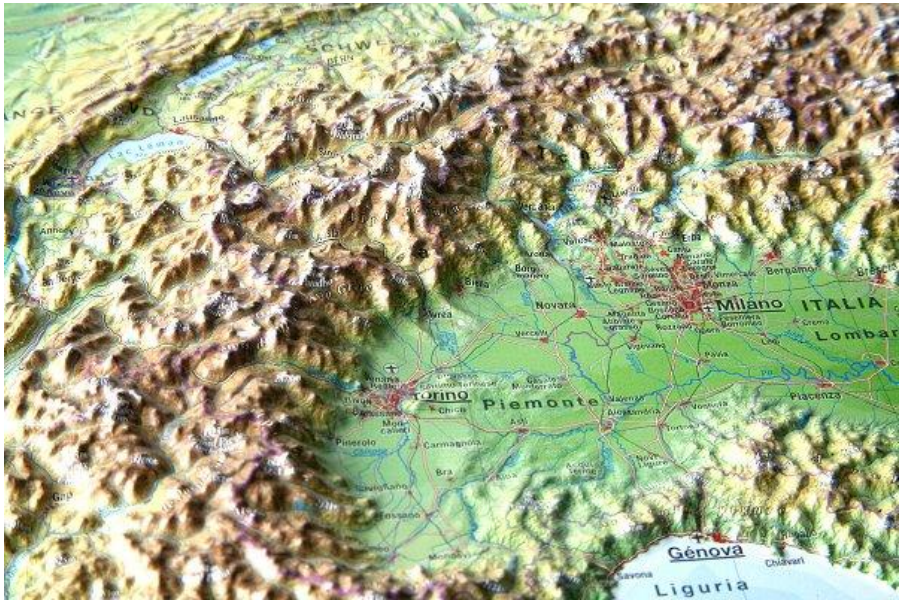


A bit of model theory……



A 3D relief model of the alps....



is useful to identify major ridges, passes and valleys
is useful to identify potential road connections
is useful to identify rivers and watersheds

is NOT USEFUL to practice hiking or climbing
is NOT USEFUL to find the safest roads
is NOT USEFUL to learn about alpine culture

“All models are wrong, but some are useful”.
George E. P. Box

A bit of model theory……

models are not the real world, but simplified versions of it that only focus on the **things we want to study** in a given situation

A model is always linked to a question. It is a „setup“ that may answer this specific question (not any question)

The term „model“ is often used sloppily, mixed with colloquial language and non-scientific thinking. The term „test method“ is more useful.

Neither animal nor cells are a test method; both can be an element of a test method

Additional elements of a test method:

Exposure scheme

Endpoints

Prediction model

Purpose definition

Purpose(s) of a test method for DNT……

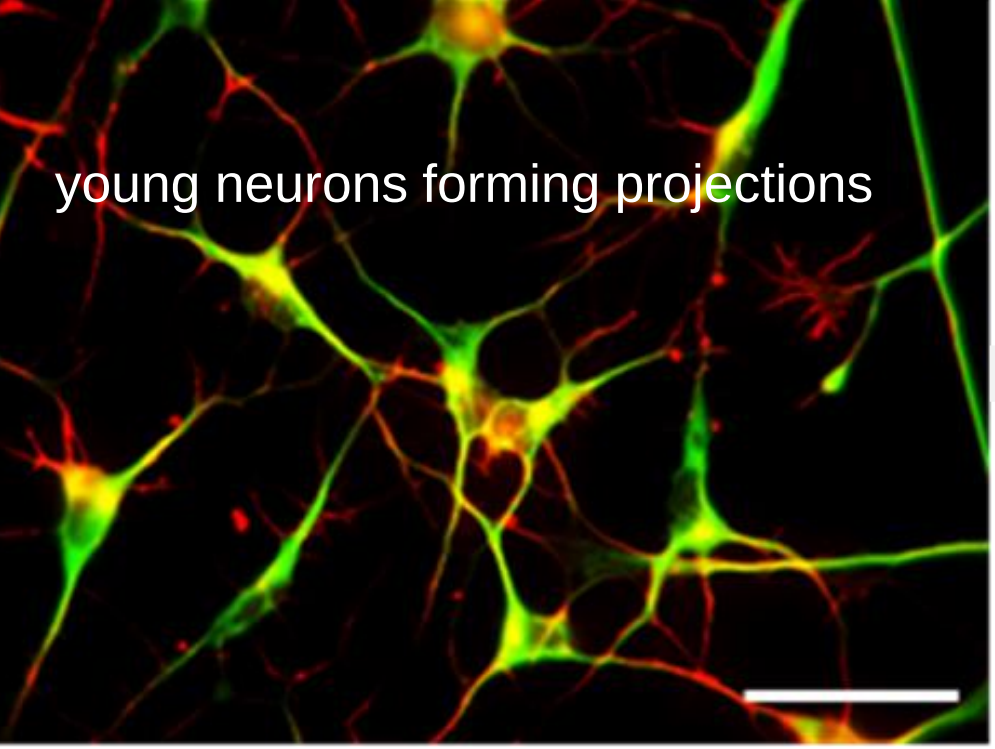
Prediction of chemicals that trigger e.g. language delay (qualitative or quantitative)?

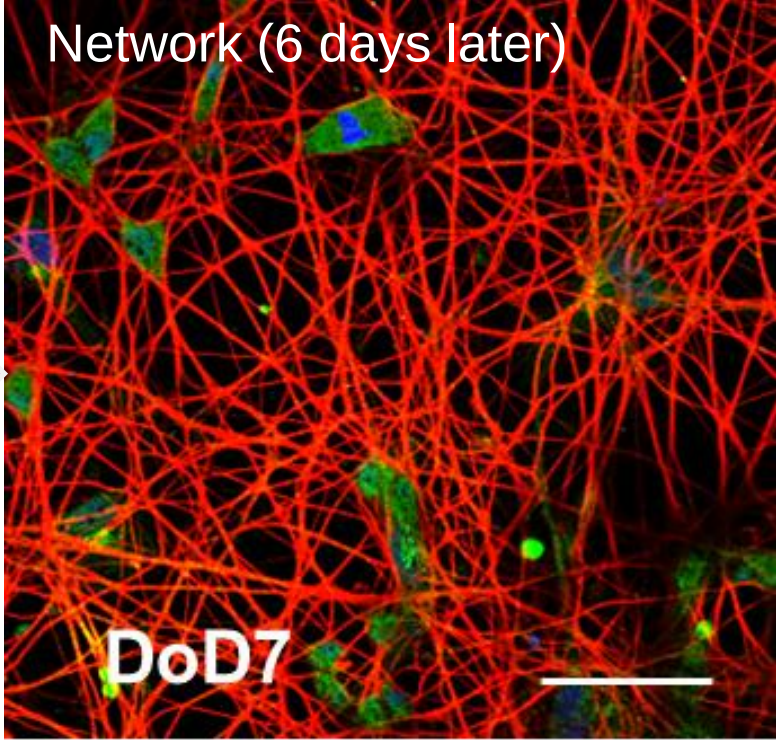
Prediction of chemicals that trigger any kind of brain function impairment (qualitative or quantitative or ranking)?

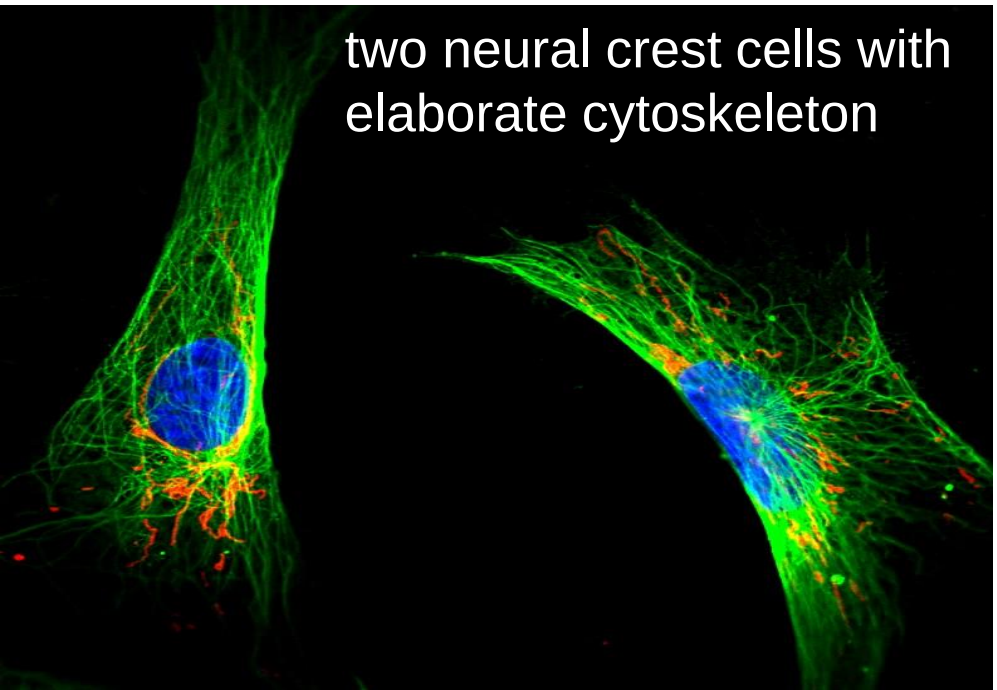
Prediction of a chemical‘s minimum concentration/dose that triggers brain function impairment?

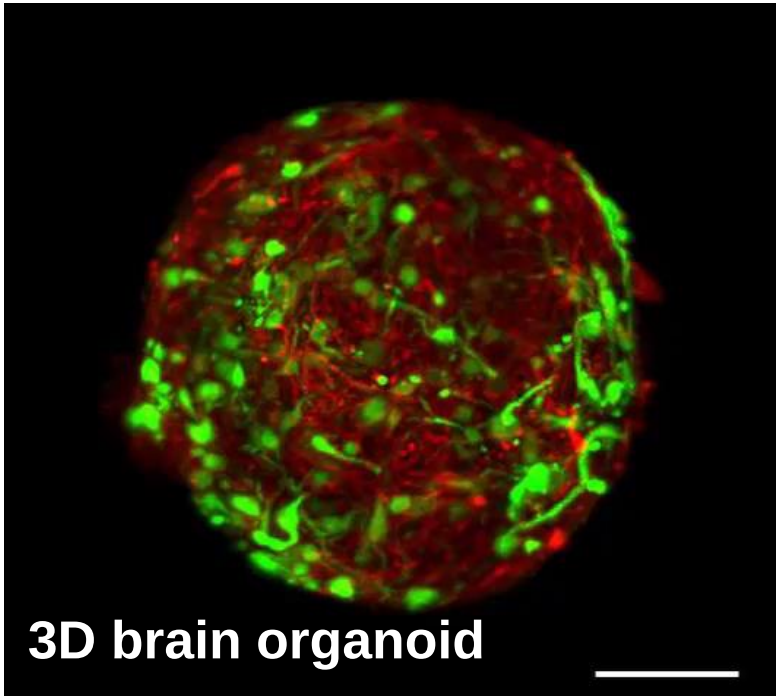
Prediction of a chemical‘s maximum concentration/dose that does not trigger brain function impairment?

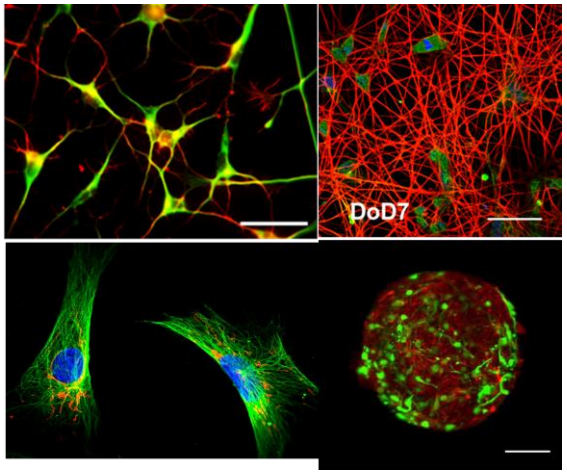
Prediction of the likelihood of a chemical to trigger brain function impairment at a given exposure/internal concentration?


young neurons forming projections


Network (6 days later)
DoD7

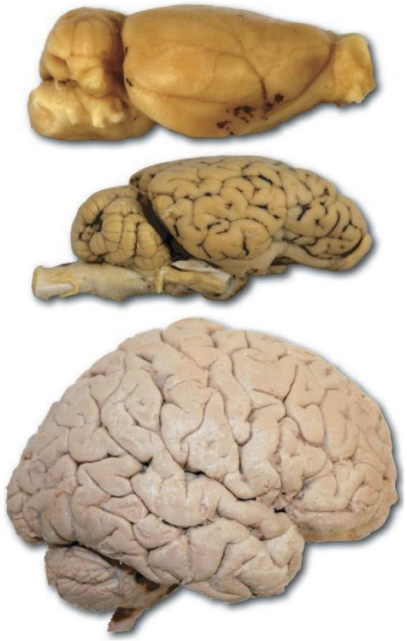

two neural crest cells with
elaborate cytoskeleton


3D brain organoid



Advantage: human cells (biochemistry)
Disadvantage: function/predictivity
little explored

Advantage: long experience
Disadvantage: no human cells



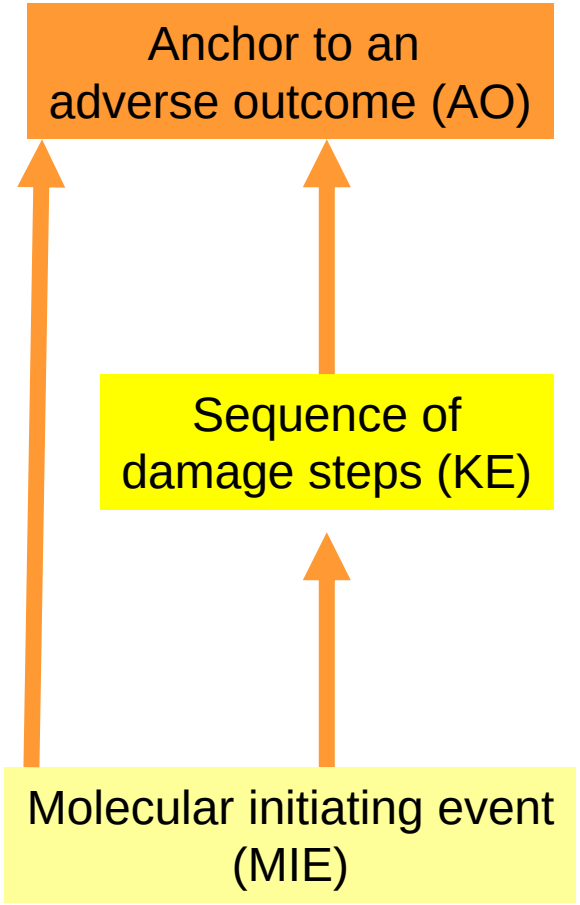
rat

sheep

human

Approaches to use functional testing for hazard assessment

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



1. Damage assessment,
but using information from earlier stages

2. Non-damage assessment:
→ no „footprints“ / „hints“ leading to damage detectable
(even after a comprehensive search)

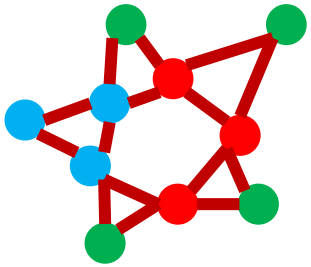
How would one define „no damage“?

No change in brain connectivity

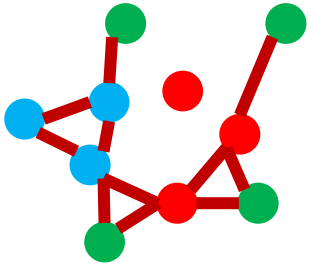
Connectivity is structural (e.g. axons, dendrites, synapses, cell positions&number)
Connectivity is also functional (e.g. strength of synapses, channel&receptor expression)

A mini-model brain for exemplification:

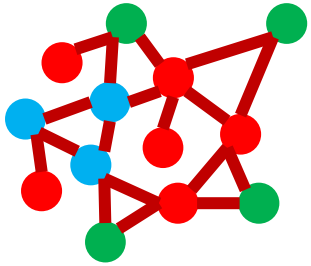
How is connectivity altered?



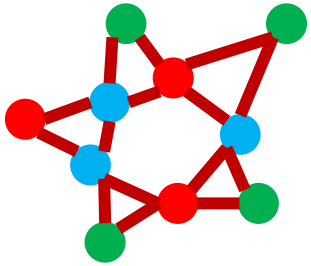
Normal / control



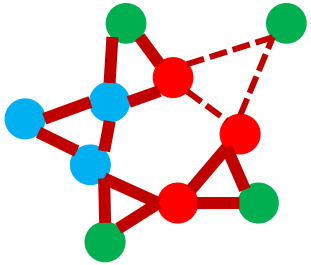
Neurite loss



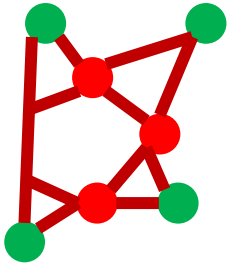
Hyperproliferation



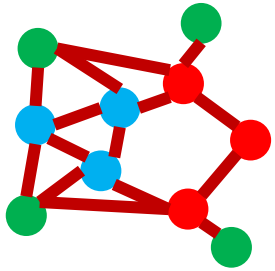
Failed Differentiation



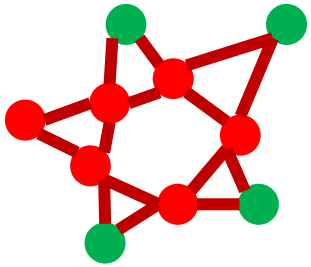
Synaptic defects



Cell death / Hypoproliferation



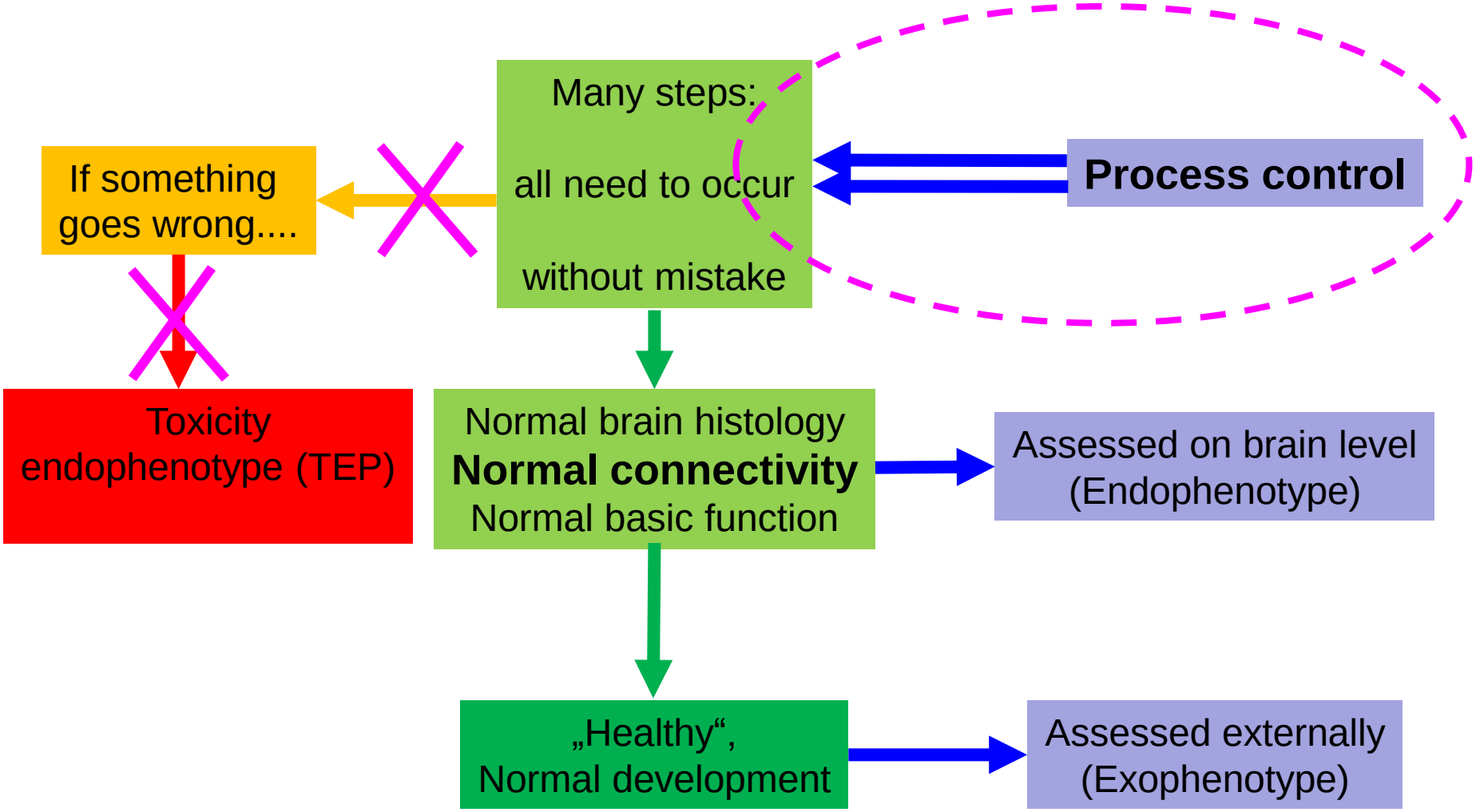
Migration defect/Disarray



Lost subpopulation

Approaches to use functional testing for hazard assessment

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



Aschner et al. (2017) ALTEX 34:49-74; Bal-Price et al. (2015) Arch Toxicol 89:269-87

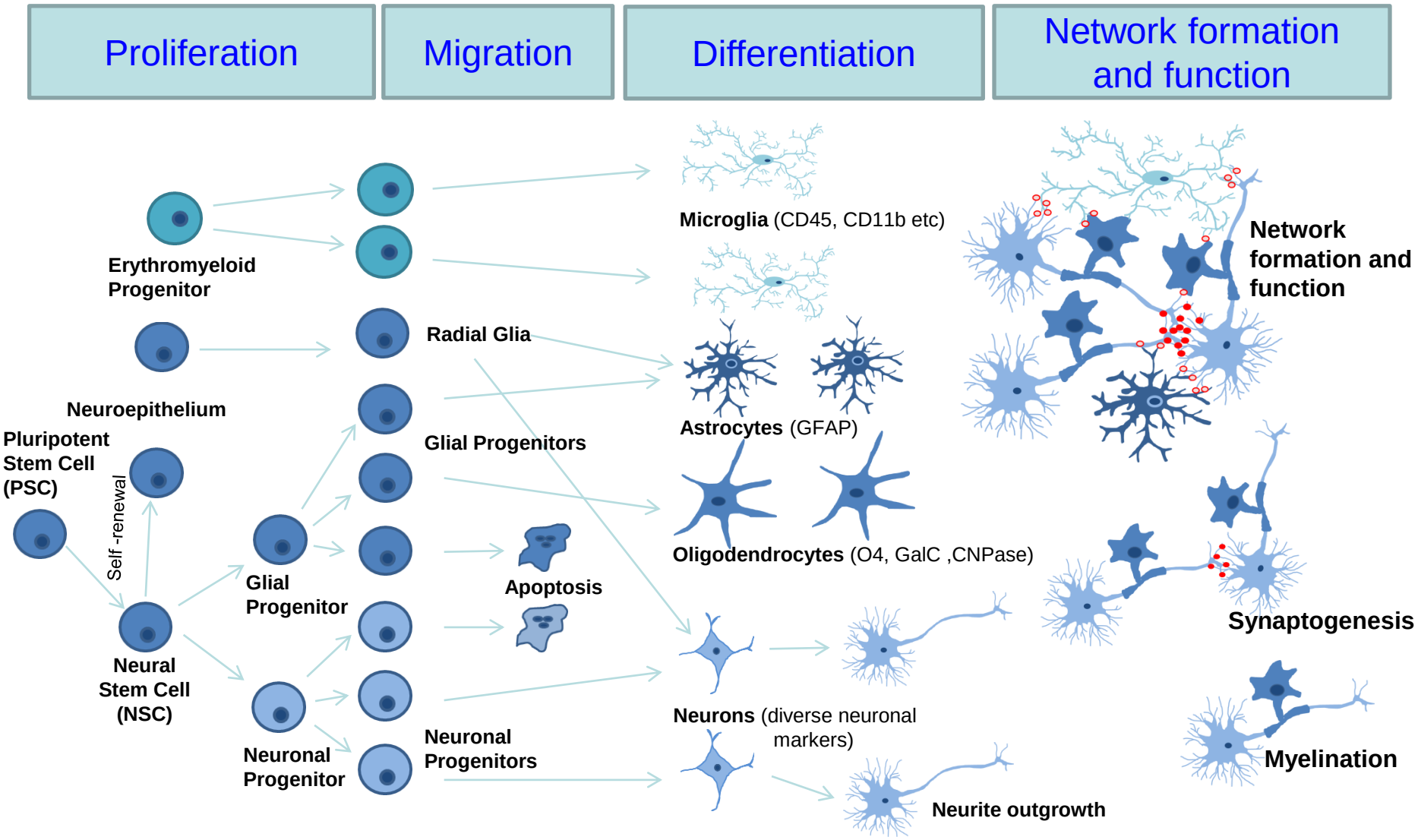
AOP-based new test principle:
‚process control' instead of ‚end stage control'

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.

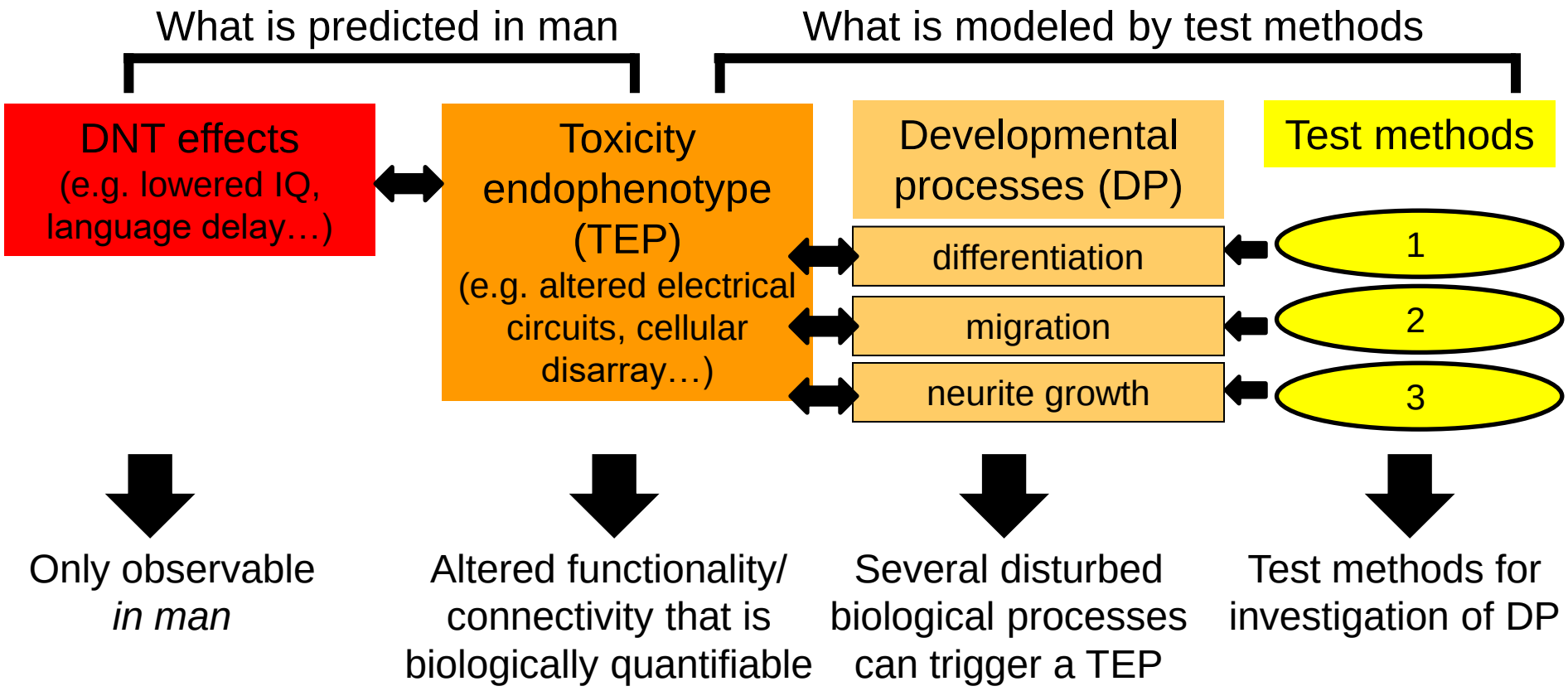
Procedure: define and establish test methods for key neurodevelopmental processes and evaluate interference by test chemicals

Key neurodevelopmental processes



Bal-Price (2018) ALTEX

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

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

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

Implications of the novel testing approach

Predictions are made on „potential“ hazard, assuming that disturbance of a fundamental neurodevelopmental process WILL ALWAYS be hazardous
→conservative; not accounting for plasticity; precautionary

Confidence must be gained that ALL fundamental processes are covered, to make statement on non-toxicity

Predictions are on hazard (level of toxicity endophenotypes / disturbed connectivity), but not on type of hazard (e.g. language delay)

More suitable to predict, which concentration of a chemical is (DNT) safe; less suitable to predict type of adversity

Are animal based test methods any better?

Can one interpret / relate to human adversity.....?

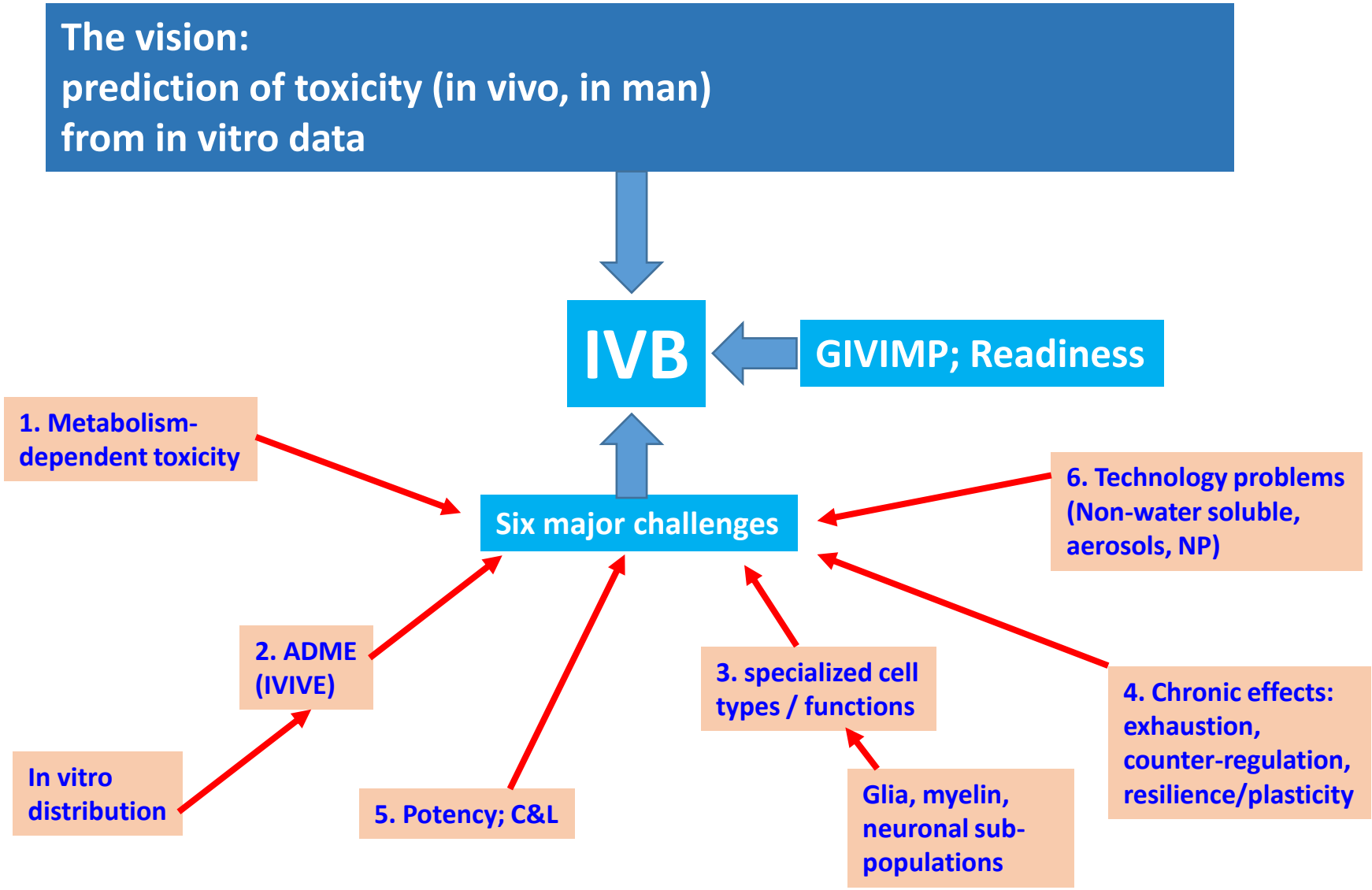
- Delayed eye opening
- Altered brain weight
- Altered light-dark preference
- Stereotypes, like increased grooming behaviour
- Altered gait on beam walking
- Altered maze performance
- Etc

In REALITY, data from animal-based tests at best indicate that SOMETHING is different from normal.
Language delays, etc: **cannot be predicted!**

➡ Animal endpoints are only indirect measures of toxicity endophenotypes

➡ Animal endpoints cannot usually be translated to human adversities

➡ **We now get this information without animals, using human cells**


The vision:
prediction of toxicity (in vivo, in man)
from in vitro data
IVB
GIVIMP; Readiness
1. Metabolism-dependent toxicity
Six major challenges
6. Technology problems (Non-water soluble, aerosols, NP)
2. ADME (IVIVE)
3. specialized cell types / functions
4. Chronic effects: exhaustion, counter-regulation, resilience/plasticity
In vitro distribution
5. Potency; C&L
Glia, myelin, neuronal sub-populations

Acknowledgement