NTP
National Toxicology Program

Developmental Neurotoxicity (DNT) Health Effect Innovation (HEI)

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National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting
June 18, 2019

Overview

- Program Management Team
- DNT-HEI Aims
- DNT Public Health Problem
- Environmental Links to Neurodevelopmental Disorders
- DNT History and Testing Challenges
- DNT Modeling Opportunities and New Framework
- DNT Screening, *In Vivo* and Translational Strategies

DNT-HEI Program Management Team

Mamta Behl

Laura Hall

Christopher McPherson

Nisha Sipes

Robert Sills

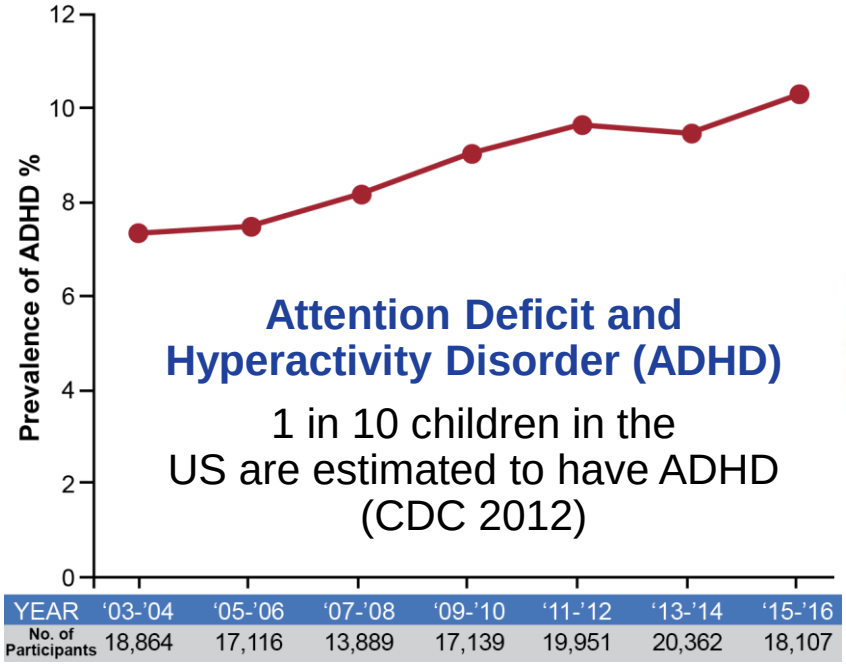
Developmental Neurotoxicity-HEI Aims

- Have global public health impact by identifying environmental chemicals that have the greatest potential to affect susceptible populations (developing embryo/fetus, infants, children)
- Provide a forum for collaborations among NIEHS scientists, NIH, EPA, and FDA's National Center for Toxicological Research; engagement with external stakeholders, including clinicians and children health advocacy groups
- Training the next generation of scientists on all aspects of neurodevelopmental disorders related to environmental exposures and on the very latest technology

Public Health Problem

Increasing prevalence of learning and behavioral disabilities and neurodevelopmental disorders in children

Sources: Pediatrics, 128(5):1007-1022, 2011; American Psychiatric Associations, 2013



YEAR	'03-'04	'05-'06	'07-'08	'09-'10	'11-'12	'13-'14	'15-'16
No. of Participants	18,864	17,116	13,889	17,139	19,951	20,362	18,107

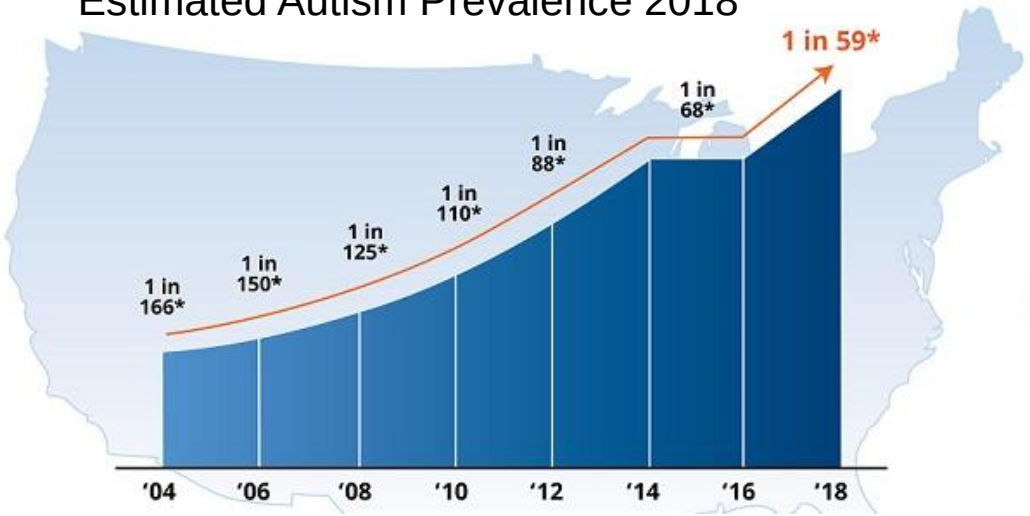
Attention Deficit and Hyperactivity Disorder (ADHD)

1 in 10 children in the US are estimated to have ADHD (CDC 2012)

Sources: abcnews.go.com, "ADHD rates in kids have increased over the past 20 years, new study says"; Journal of the American Medical Association (JAMA)

Autism Spectrum Disorder

Estimated Autism Prevalence 2018



* Centers for Disease Control and Prevention (CDC) prevalence estimates are for 4 years prior to the report date (e.g. 2018 figures are from 2014)

Source: autismspeaks.org, "CDC increases estimate of autism's prevalence by 15 percent, to 1 in 59 children"

- Economic costs associated with neurodevelopmental disorders is staggering, estimated to be approximately $461 billion by 2025

Project TENDR: Targeting Environmental Neuro-Developmental Risks. The TENDR Consensus Statement

http://dx.doi.org/10.1289/EHP358

- Leading scientists came together to issue a call to action to reduce widespread exposures to chemicals that interfere with fetal and children's brain development.

- Summarized environmental links to neurodevelopmental disorders:
  - Organophosphate pesticides
  - Polybrominated diphenyl ether flame retardants
  - Combustion-related air pollutants
  - Phthalates
  - Lead
  - Mercury
  - PCBs

Source: Environmental Health Perspectives, July 2016, Vol. 124, No. 7

Environmental Links to Neurodevelopmental Disorders

- A goal of the DNT-HEI program is to develop a new framework for identifying environmental chemicals that can contribute to neurodevelopmental disorders and thereby reduce the potential for learning disabilities in our future generation.

Historical Perspective – In Vivo DNT Testing Strategies

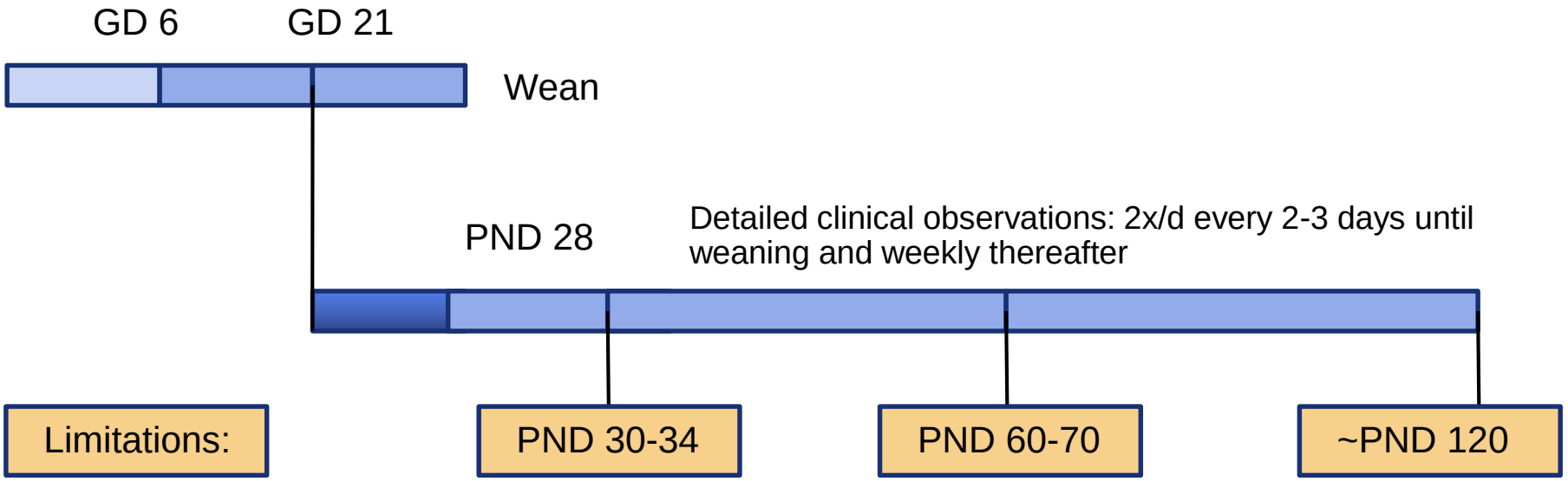
- Stand alone DNT study
- Created guidance document for DNT studies

- DNT studies incorporated into perinatal inhalation and reproductive studies

- Conducting and reporting studies

2003-2011 2012 2013 2014 2015 2016 2017 2018

DNT Study Design- Continuous Exposures



Limitations:

- Number of DNT studies conducted:4

PND 30-34

Motor activity
Motor Function
Motor and sensory function

PND 60-70

Motor activity
Motor Function
Motor and sensory function
Morris water maze

~PND 120

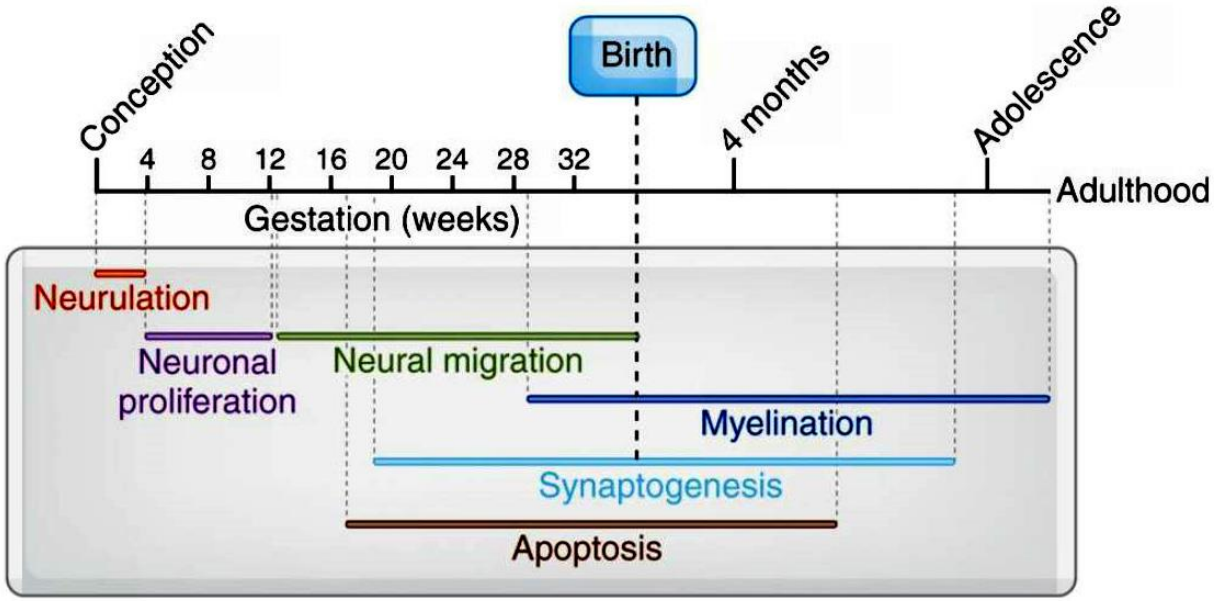
Brain weights
Neurohistopathology
(CNS/PNS)

Challenges:

- DNT studies resource intensive-time, money and number of animals
- Limited potential for understanding mechanisms of neurodevelopmental disorders

DNT Modeling Opportunities

Understanding mechanisms linked to major neurodevelopmental process



Timeline of Major Events in Human Brain Development

- NTP is developing a battery of *in vitro* assays anchored to key neurodevelopmental processes for evaluating DNT effects

Source: Tan, G.Z., et. al., Neuropsychopharmacology Reviews, 5:147-168, 2010

There is a Need for a New Framework for Assessing DNT

- Rapid and balanced assessment strategy for evaluating behavior, structural and chemical disruption, and understanding mechanisms of DNT

Project TENDR Consensus:
Targeting **E**nvironmental
Neuro-**D**evelopmental **R**isks.
Source: http://dx.doi.org/10.1289/EHP358

Toxicology and Applied Pharmacology 354 (2018) 3–6

Contents lists available at ScienceDirect

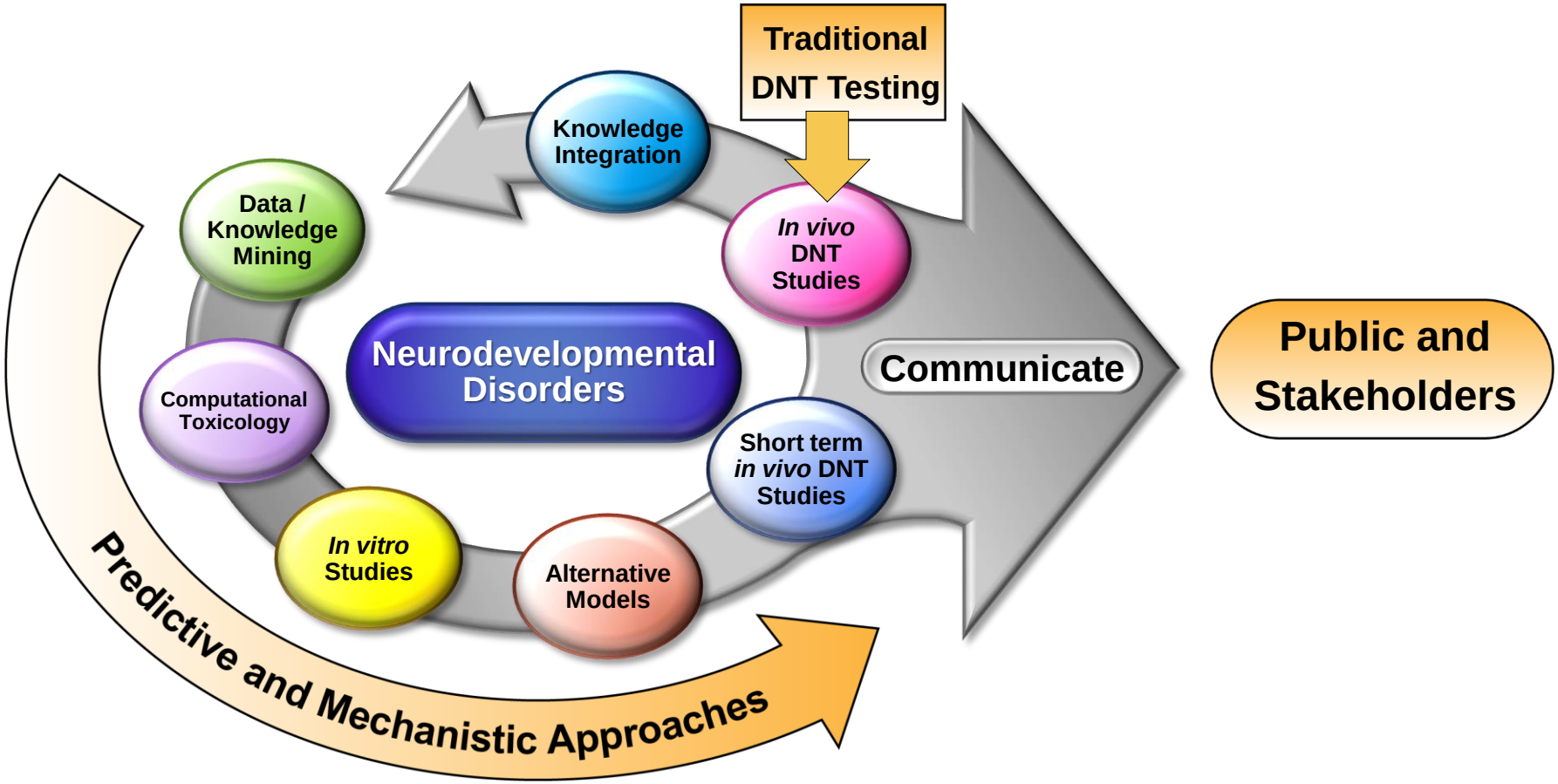
Toxicology and Applied Pharmacology

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

Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes

DNT-HEI New Framework

- Integrated Translational Toxicology Pipeline is key to addressing DNT public health problems
- Applying our capabilities in deliberate, integrated and complementary ways



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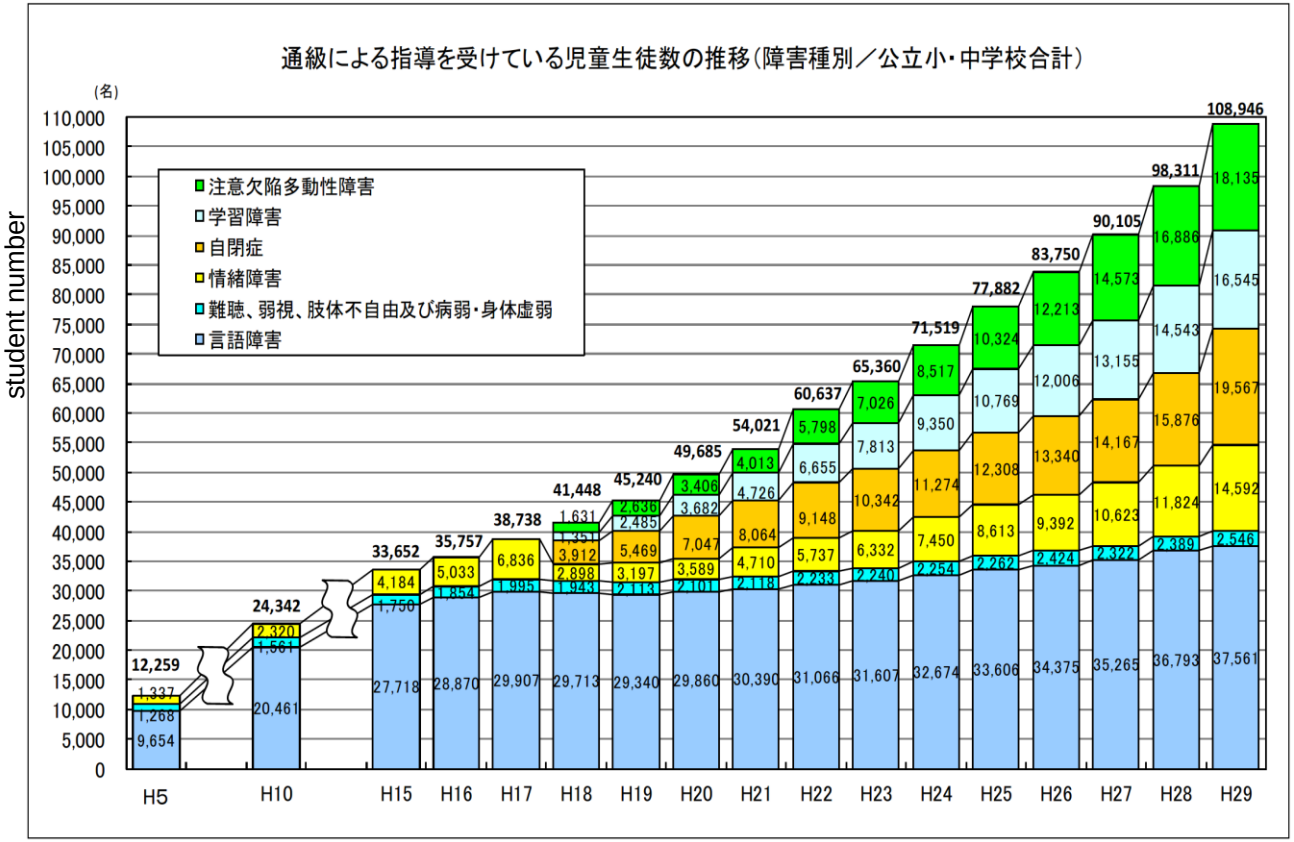
Global Rise in Neurodevelopmental Disorders

Official report from Japanese Ministry of Education, 2017

http://www.mext.go.jp/a_menu/shotou/tokubetu/__icsFiles/afieldfile/2018/05/14/1402845_03.pdf



Attention deficit hyperactivity disorder

Learning disability

Autism

Emotional disorder

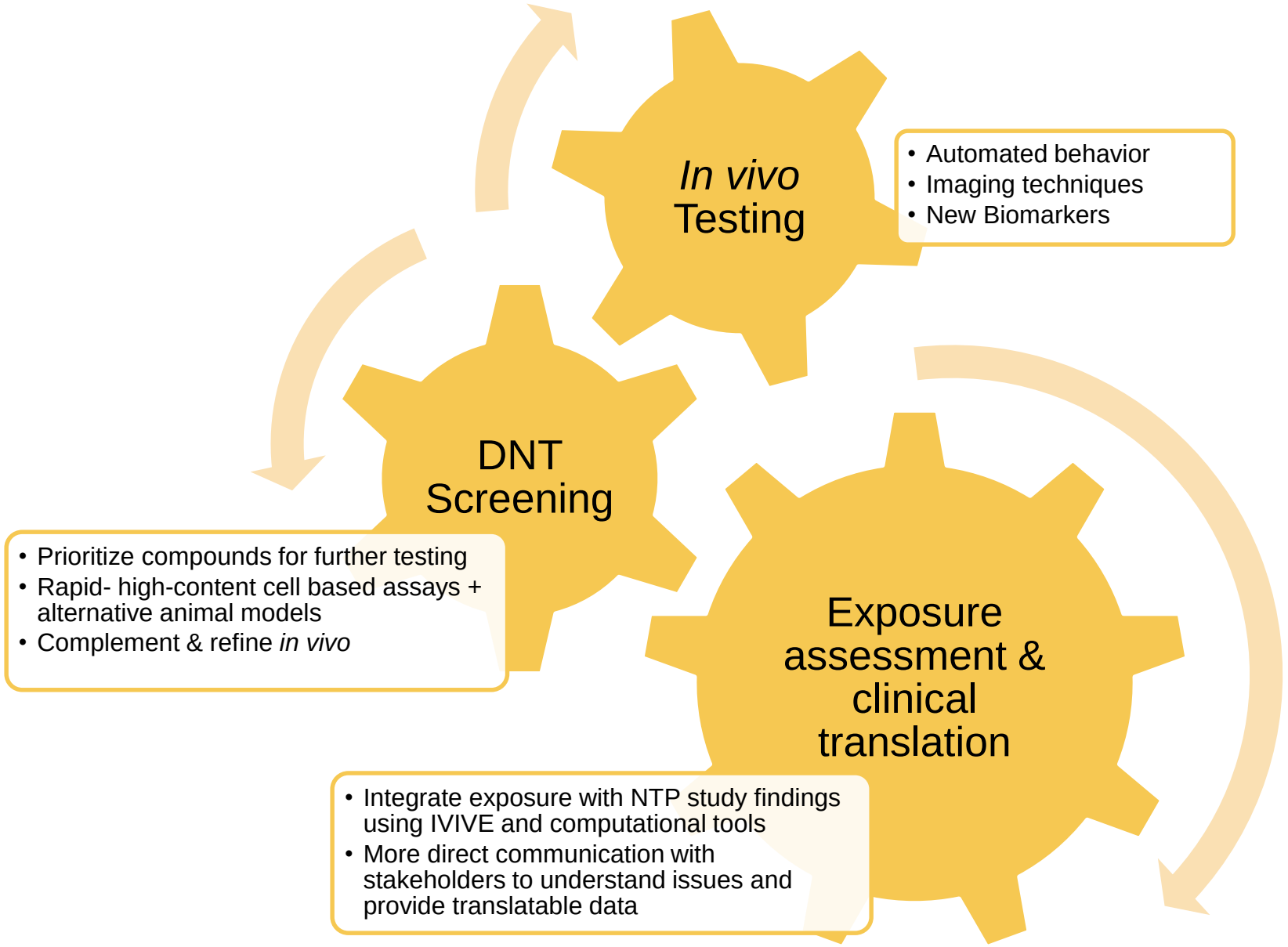
Hearing, Amblyopia, etc

Speech disorder

1993 1998 2003 2006 2017

Slide Courtesy: Kentaro Tanemura

NTP's Integrative Testing Strategy for DNT



DNT Screening

DNT Screening: What have we done so far?

(Not so) Historic Perspective

Stand-alone DNT study

Created guidance document for DNT studies

- DNT studies incorporated into perinatal inhalation and reproductive studies

- Conducting and reporting studies

2003-2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018

Created library to screen assays targeting neurodevelopmental processes

First NTP paper published on concept and application of battery for DNT screens

Workshop: Integrated Testing Strategies for DNT

Updated BSC on workshop goals and outcomes

Updated BSC on interactive web-based tool (DNT-DIVER)

DNT Screening: Data Integration & Visualization Tool

- The NTP created a free, publicly available interactive web-based tool known as DNT- DIVER
  - **D**evelopmental **N**euro**T**oxicity **D**ata **I**ntegration and **V**isualization **E**nabling **R**esource
- First data integration and visualization resource that allows for a comparison of chemical effects across assays that cover critical nodes of neurodevelopment
- Created due to the recognition that neurodevelopment is complex; one assay will not be sufficient to address the complexity of the nervous system
  - Need for a battery

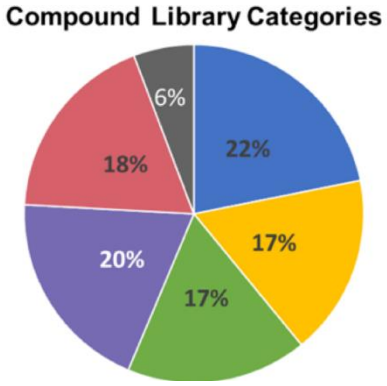
https://sandbox.ntp.niehs.nih.gov/neurotox/

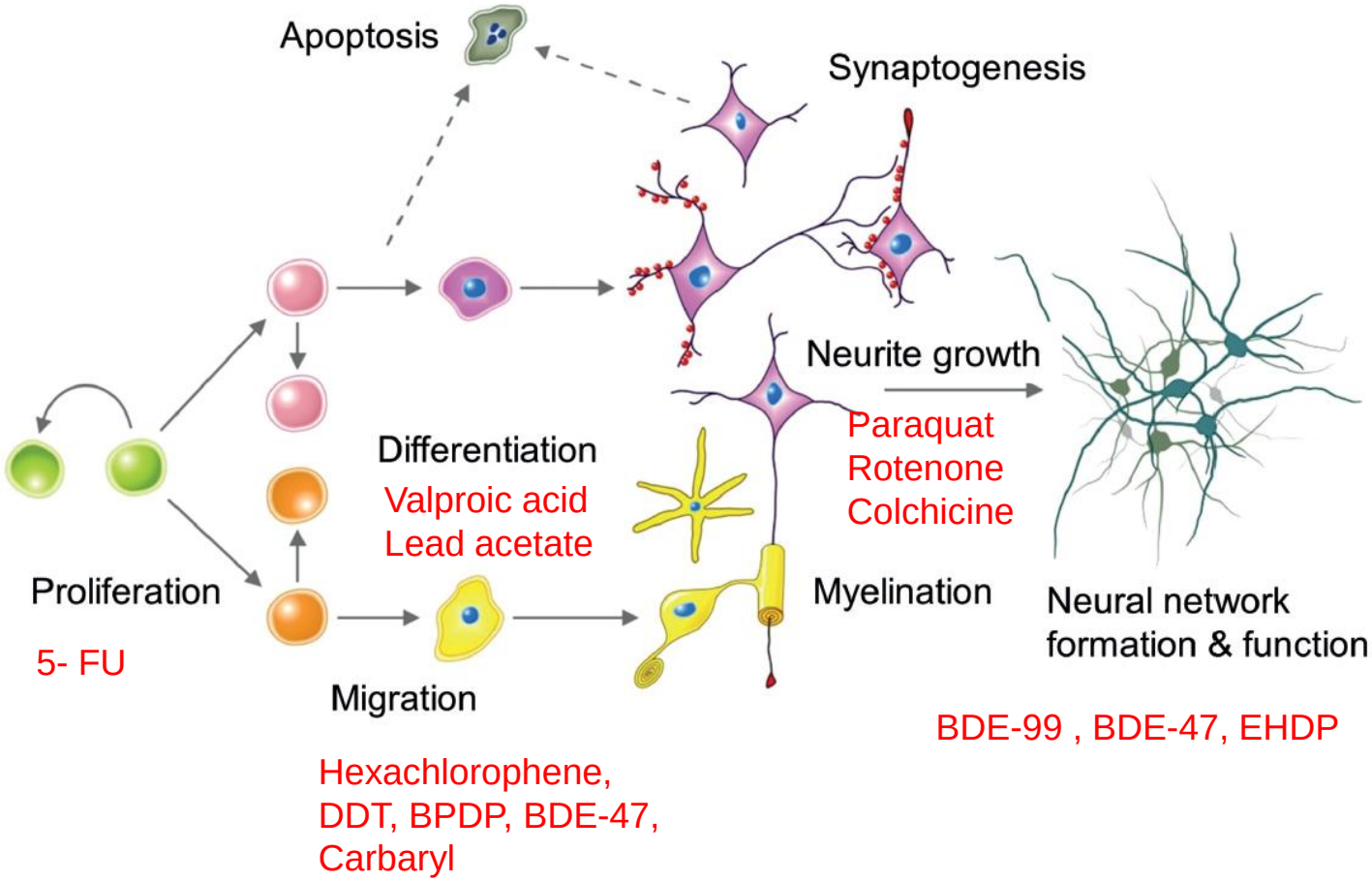
DNT Screening: What questions can we now address?

- How do compounds/ classes with unknown DNT potential look across a battery of assays?
  - Overlapping vs unique assays
- How do compounds/ classes look within an assay?
  - QSAR vs Biology
- How does data with the knowns compare with that found from in vivo studies?
  - Can this method actually be used to prioritize compounds?

Current *In Vitro* Coverage in DNT Screening

Human-derived high content, cell-based functional assays



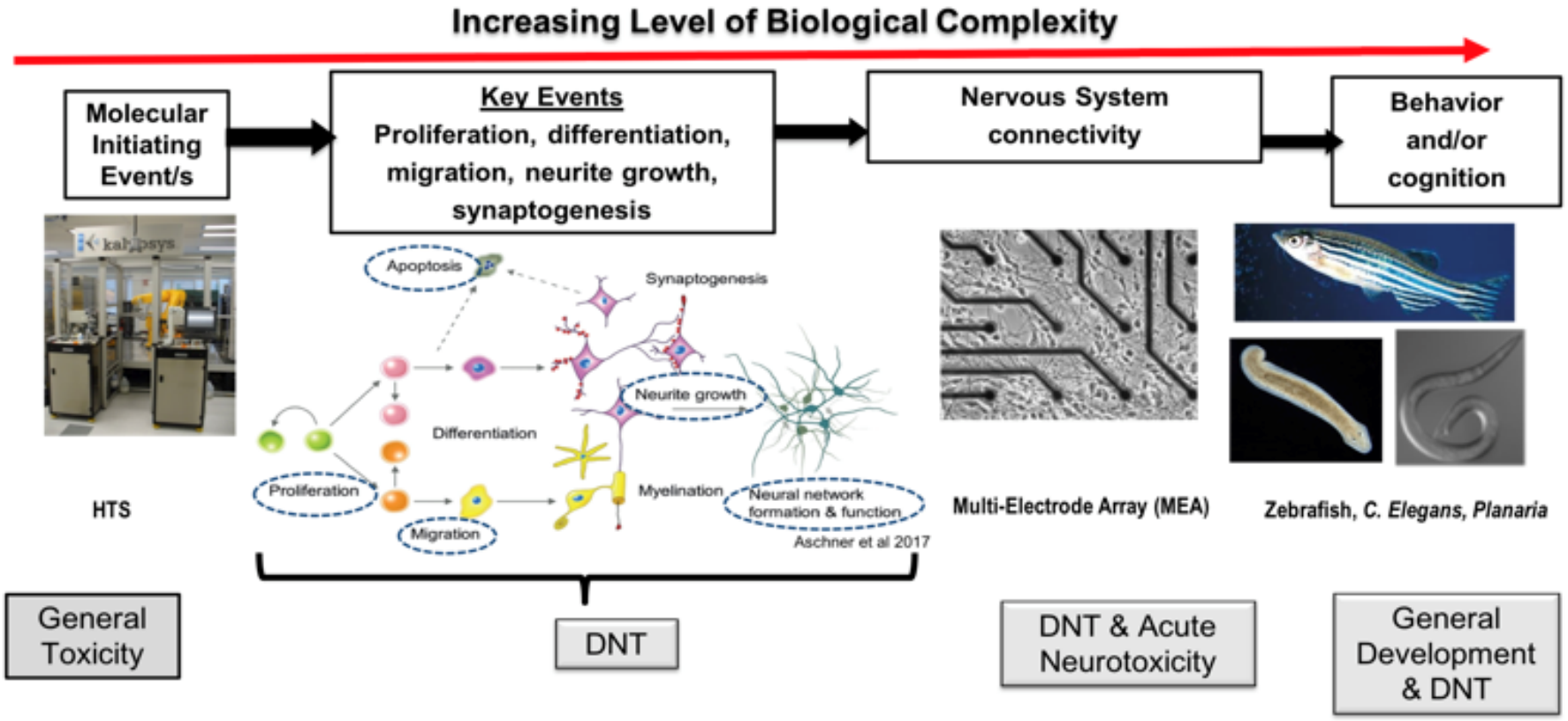


Aschner et al., 2017 & Mundy

Current Alternative Model Coverage in DNT Screening

- Behavior in complementary animal models
  - Zebrafish: Motor activity
    - Other assays being evaluated in lower throughput systems (startle, L&M)
  - Planaria: gliding and swimming, phototaxis, thermotaxis, and scrunching

Creation of a DNT Screening Battery



Most of these fall under “high readiness "criteria as recently determined by OECD (OECD 2017)

For further details..

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‡

ToxSci,167 (1), 2019, 6-14

For further details..

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Screening for Developmental Neurotoxicity at the National Toxicology Program: The Future is Here
Mamta Behl, Kristen Ryan, Jui-Hua Hsieh, et al.

Comparative Analysis of Zebrafish and Planarian Model Systems for Developmental Neurotoxicity Screens Using an 87-Compound Library
Danielle Hagstrom, Lisa Truong, Siqi Zhang, Robert Tanguay, and Eva-Maria S. Collins

Multi-Behavioral Endpoint Testing of an 87-Chemical Compound Library in Freshwater Planarians
Siqi Zhang, Danielle Hagstrom, Patrick Hayes, Aaron Graham, and Eva-Maria S. Collins

International Regulatory and Scientific Effort for Improved Developmental Neurotoxicity Testing
Magdalini Sachana, Anna Bal-Price, Kevin M. Crofton, et al.

Functional and Mechanistic Neurotoxicity Profiling Using Human iPSC-Derived Neural 3D Cultures
Oksana Sirenko, Frederick Parham, Steven Dea, et al.

Teratological and Behavioral Screening of the National Toxicology Program 91-Compound Library in Zebrafish *(Danio rerio)*
Katharina Dach, Bianca Yaghoobi, Martin R. Schmuck, et al.

DNT-DIVER

National Toxicology Program
U.S. Department of Health and Human Services

About | Datasets | Quality Control | Concentration Response | BMC by Lab | Integrative Analyses | Resources

Developmental **N**euro**T**oxicity **D**ata **I**ntegration and **V**isualization **E**nabling **R**esource (**DNT-DIVER**)

Research shows that a child's developing nervous system is far more vulnerable to chemical exposures than an adult nervous system. Recent increases in the rise of neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD), dyslexia, and autism spectrum disorder have prompted scientific interest in the potential contribution of environment toxicants to these disorders.

Traditional animal, or *in vivo*, studies provide important information about developmental neurotoxicity (DNT) but they are time and resource intensive. NTP has also developed more rapid screening tools that use human cell-based, or in vitro, assays, as well as alternate animal models such as zebrafish and planaria to identify toxicants with potential for DNT. Multiple tests, or assays, are often required to represent the complexity of the developing nervous system, but that can make it challenging to compare and summarize results.

NTP designed the **D**evelopmental **N**euro**T**oxicity **D**ata **I**ntegration and **V**isualization **E**nabling **R**esource (**DNT-DIVER**) to analyze, compare, and visualize multiple DNT assays in an interactive web-application.

Within DNT-DIVER, comparisons are organized on different tabs including experimental design summary, quality control, chemical-specific concentration response curves, ranking of chemical toxicity per lab/assay, and comparison of results across assays. We also provide downloadable resources including raw and final data, along with the NTP Chemical Library (Microsoft Excel) that was tested by collaborators in their respective models.

Suggestions?

We are currently refining assays, analyses, and visualizations, and would appreciate your input! Please direct questions or suggestions to Mamta Behl.

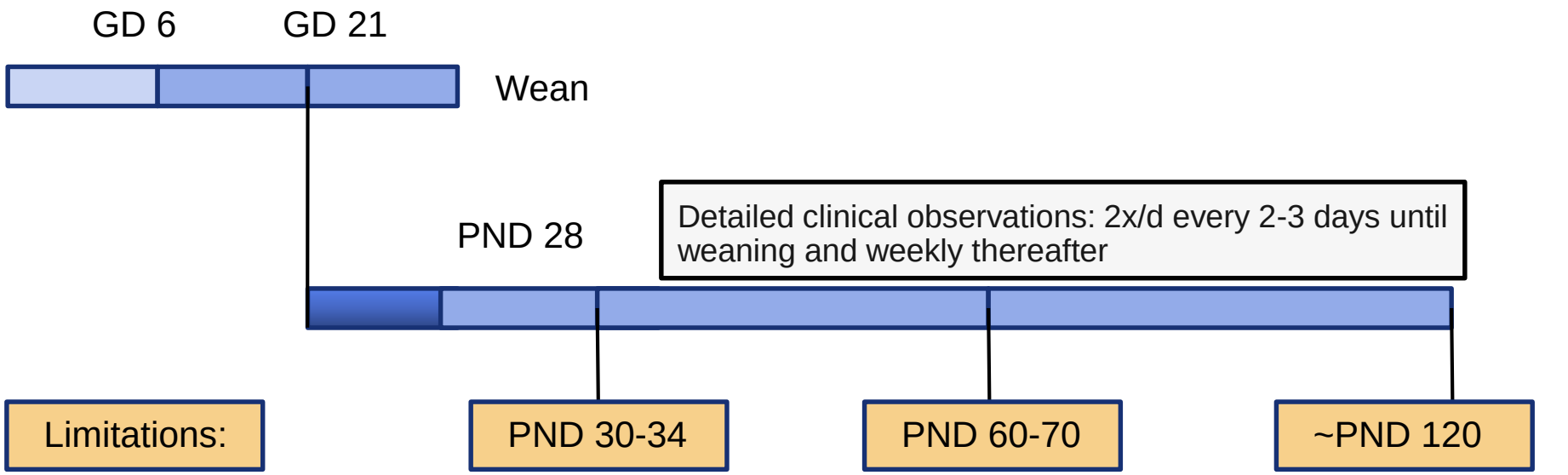
https://sandbox.ntp.niehs.nih.gov/neurotox/

DNT Screening: Where to from here?

- Plan on building on our data-base and expanding to:
  - Incorporate more chemicals
  - Improve assay coverage (3-D models, mixed culture, barriers)
  - Evaluate status of current assays
- In parallel working on lower throughput, relevant assays
  - Genetically diverse mouse and humans lines

In vivo DNT Testing

Current *in vivo* Design



Limitations:

- Number of DNT studies conducted:4

Motor activity
Motor Function
Motor and sensory function

Motor activity
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Morris water maze

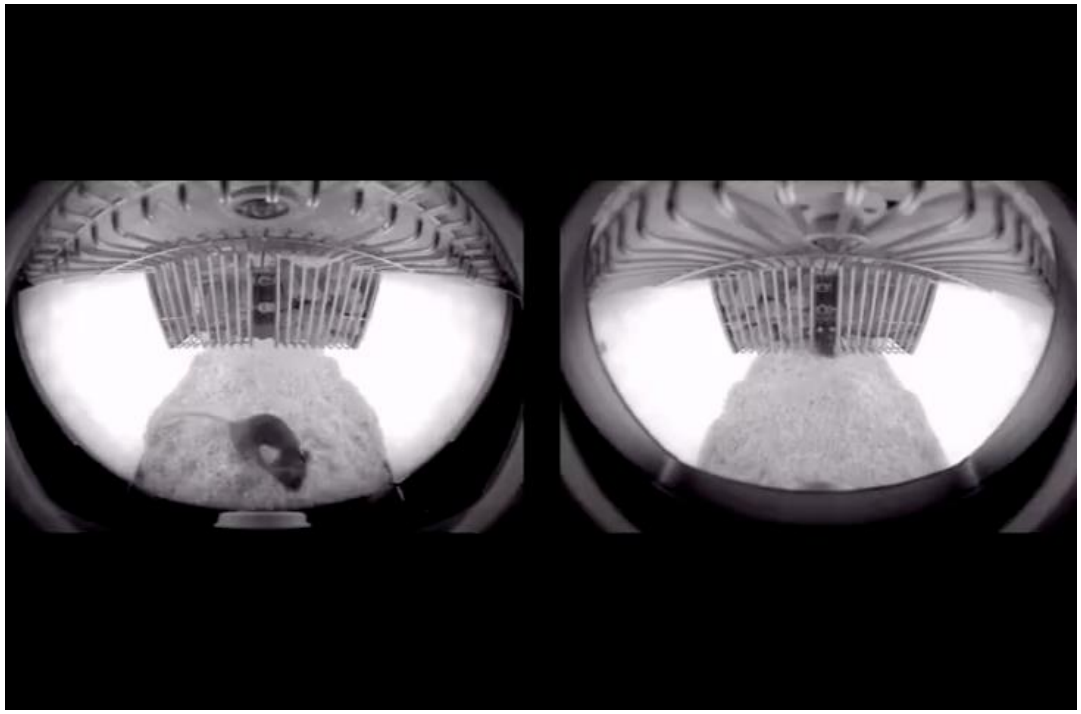
Brain weights
Neurohistopathology
(CNS/PNS)

Challenges:

- DNT studies resource intensive-time, money and number of animals
- Limited potential for understanding mechanisms of neurodevelopmental disorders

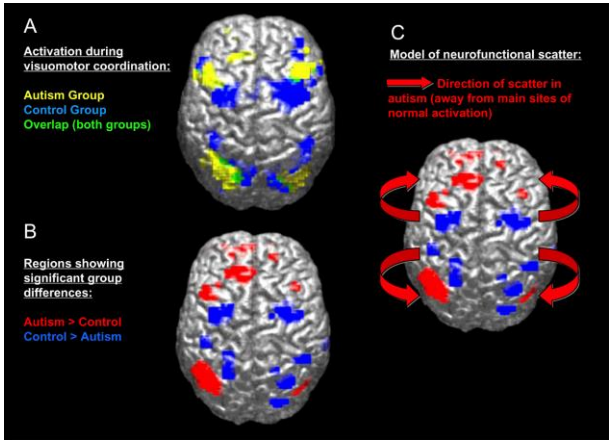
In vivo DNT testing: Where to from Here?

- Capture comprehensive information from same/ lesser animals by continuous monitoring
  - Overlay with artificial intelligence



In vivo DNT testing: Where to from Here?

- Incorporate imaging tools (MRI)
- Clinically relevant biomarkers of brain injury



More comprehensive & objective
Reduce time & number of animals
Improve translation

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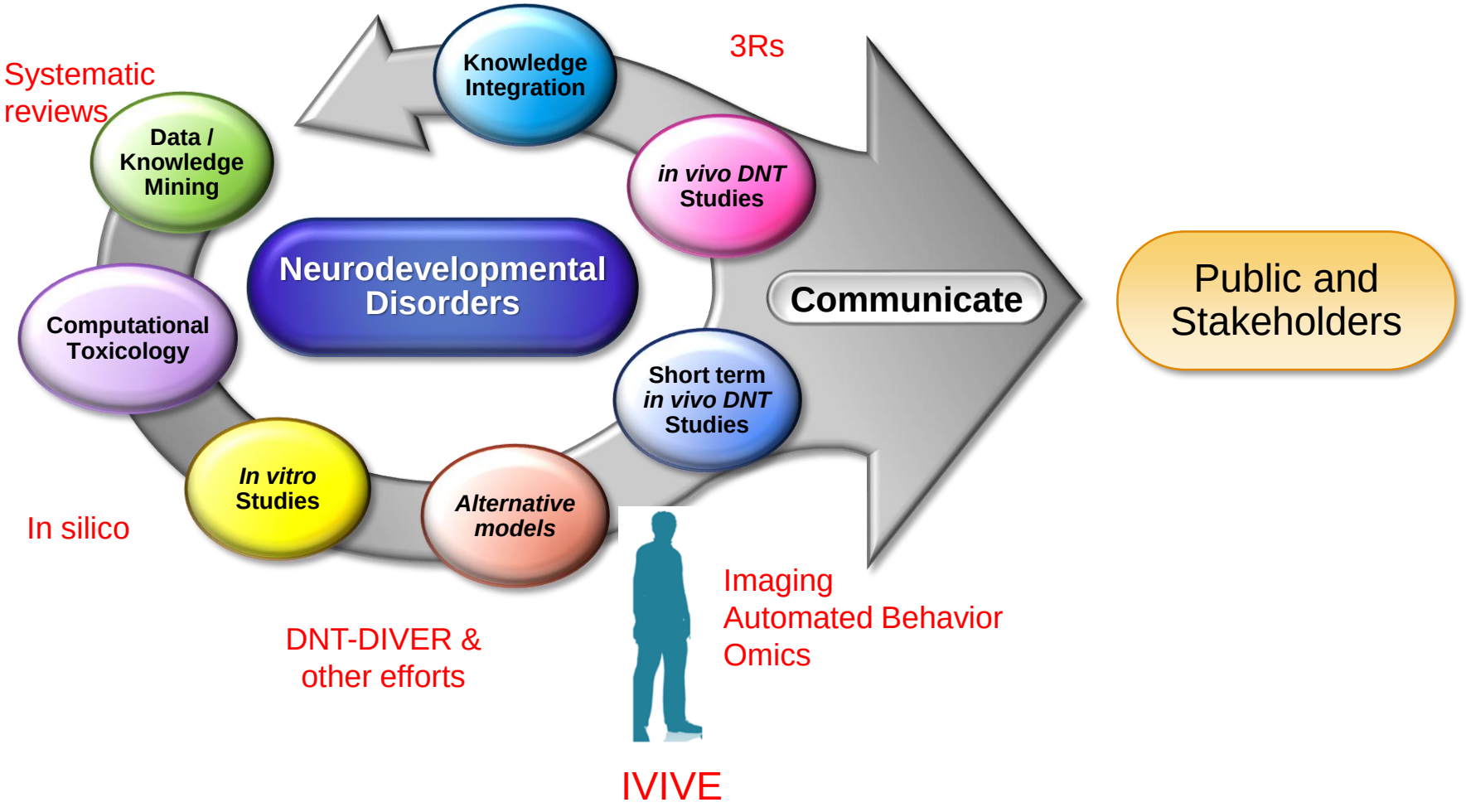
Exposure Assessment & Clinical Translation

Translation & Communication

- Incorporation of tools to contextualize exposure with toxicity noted in our studies
  - Models to inform *in vitro in vivo* extrapolation (IVIVE)
  - Use of *in silico* approaches for data profiling
- Engage trans- NTP groups on data mining/ systematic evaluation of literature
- Engage with stakeholders relatively early on during problem formation and continue communication throughout process
  - Cohorts on exposure assessments, clinicians, collaborative expert groups (e.g. TENDR)

Translational Toxicology Pipeline

Applying our capabilities in deliberate, integrated and complementary ways.



What do we ultimately hope to accomplish?

- Improve methods to identify compounds in the environment with unknown neurotoxic potential
- Provide data for timely public protection
- Better understand how *in vitro* studies can inform evaluation in animals & humans

Questions and Comments