Swiss 3R Competence Centre

What's New in REDUCTION REPLACEMENT REFINEMENT of Animal Use in Research?

Advancing Alternatives to Animal Models in Neuroscience

Neuroscience, the study of the brain and nervous system, is one of the research areas in Switzerland that has relied heavily on the use of animals, as revealed by our analysis of trends in the number of animal uses over the last 27 years (see our animal use report).

As scientific methods advance, new possibilities are emerging to replace animal experiments and reduce the number of animals required. Central to this progress is the development of human cell-based models that offer both biological relevance and a more ethical approach to studying complex brain diseases.

Below we share the most recent work and advancements from three research groups. Together, they show how novel in vitro methods are being used to address major public health questions from evaluating whether environmental chemicals may harm the developing brain, to understanding neurodevelopmental disorders such as ADHD, and finding better ways to study aggressive childhood brain cancer.

DID YOU KNOW?

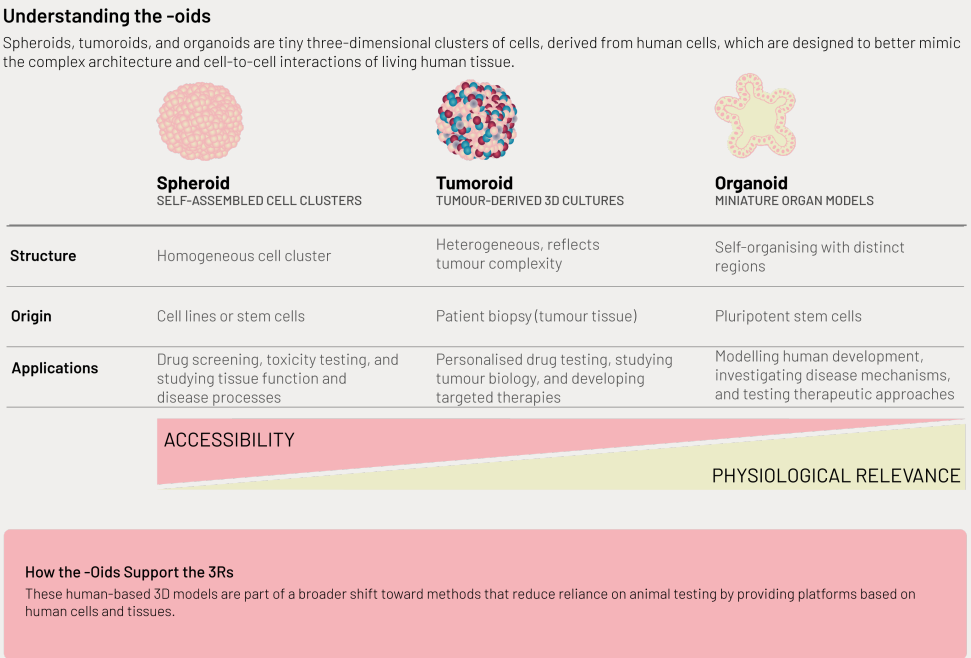
What does "in vitro" mean? In vitro (Latin for "in glass") refers to experiments conducted outside a living organism, typically in a laboratory. These methods allow researchers to study biological processes using cells and tissues in controlled conditions.

Are you familiar with the -oids?

Spheroids, tumoroids, and organoids are tiny three-dimensional clusters of cells derived from human tissue. Unlike traditional flat cell cultures, these 3D models better mimic the architecture and cell-to-cell interactions found in living human tissue.

- **Spheroids** are self-assembling clusters of cells that replicate the structure and function of small pieces of tissue, such as part of the brain.
- **Tumoroids** are grown directly from a patient's tumour biopsy, retaining the cellular diversity and characteristics of the original cancer.
- **Organoids** are miniature, simplified versions of organs grown from stem cells, allowing scientists to study human development and disease.

By providing more predictive and human-relevant platforms, these models support the **Replacement** of animal testing and the **Reduction** of animal numbers in biomedical research.



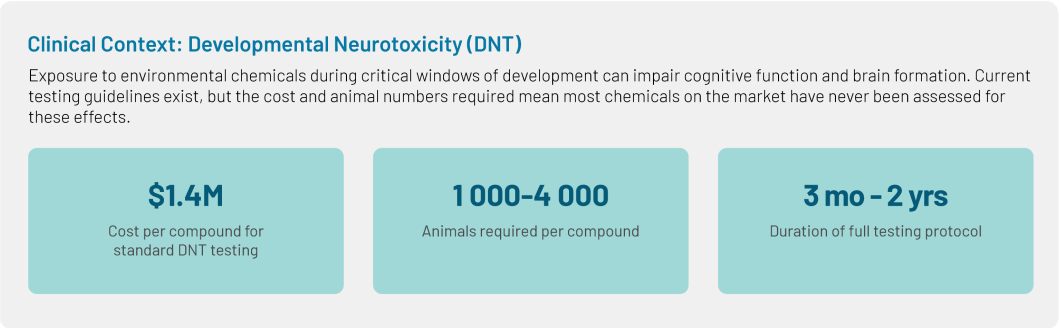
Improving Toxicology and Drug Development with Human Brain Spheroids

The work of Dr. David Pamies — *Head of the Stem Cell and Organoid Facility at the University of Lausanne.*

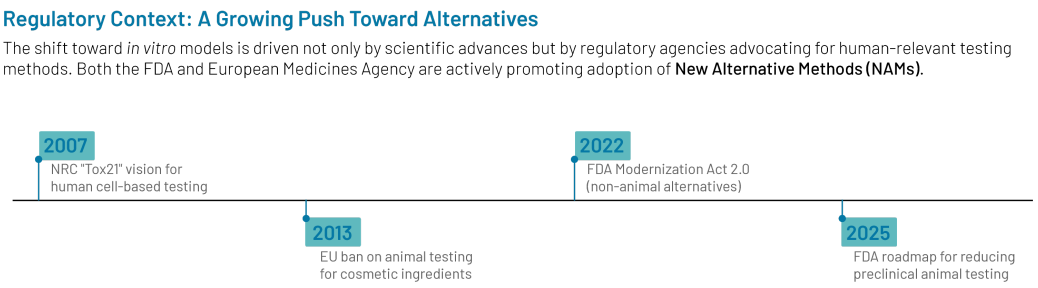
There is substantial evidence that chemicals in our environment can harm brain development, particularly during the period from early pregnancy through to around 25 years of age. Yet the vast majority of chemicals in commerce have never been tested for these effects.

The challenge

Traditional testing for developmental neurotoxicity (harm to the developing brain) requires large numbers of animals and significant resources. This creates a major barrier to routine safety testing for many chemicals.



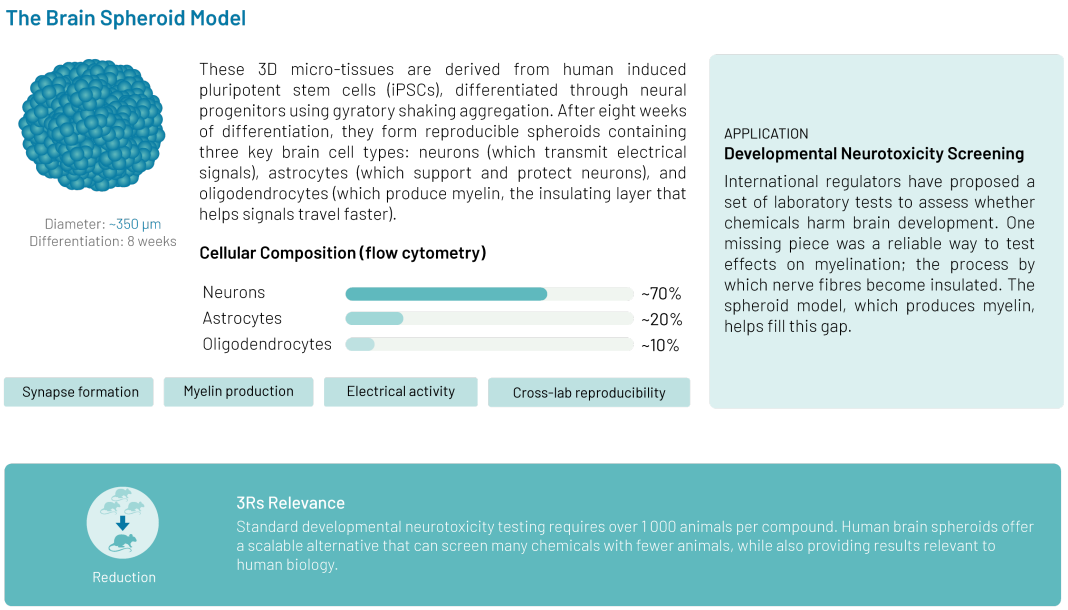
There is a significant push from regulatory bodies such as the EFSA (European Food Safety Authority), the FDA (US Food and Drug Administration) and the European Medicines Agency to adopt New Alternative Methods (NAMs). These are complementary approaches to the use of animals in research, including methods like organ-on-a-chip (small devices with channels that allow fluids to flow past human cells, simulating aspects of organs), the use of -oids, and computational models.



The 3Rs approach

Dr Pamies and team have developed human brain spheroids: tiny, 3D micro-tissues derived from human stem cells. After eight weeks of development in the laboratory, these spheroids contain three key brain cell types:

- Neurons, which transmit electrical signals
- Astrocytes, which support and protect neurons
- Oligodendrocytes, which produce myelin (the insulating layer that helps signals travel faster along nerve fibres)



The presence of myelin is particularly important. Myelination, the process of forming this insulating layer, is a critical part of brain development. Standard animal and basic cell culture models have various limitations in reproducing myelination, which makes it harder to test whether chemicals interfere with it. In this spheroid model, researchers can expose brain cells to chemicals at defined concentrations and measure effects on cell health, function and myelination under controlled conditions.

These brain spheroid models are also being applied beyond neurotoxicity testing. By co-culturing tumour tissue within the spheroid, Dr Pamies' team can test a drug's effect on both the tumour and the surrounding healthy brain tissue at the same time — particularly relevant for aggressive cancers such as glioblastoma, where a treatment's impact on healthy brain function matters as much as its effect on the tumour itself. This provides a more detailed safety profile earlier in the drug discovery process.

3Rs Relevance

While regulatory bodies have proposed a set of laboratory tests to assess whether chemicals harm brain development, a reliable way to test effects on myelination is still missing. The spheroid model from Dr Pamies' team, which produces myelin, helps fill this gap.

Human brain spheroids support Reduction by offering an alternative that allows the screening of many chemicals with fewer animals. They are particularly valuable for Replacement approaches in developmental neurotoxicity research, where differences between species in the timing and processes of brain development, such as myelination, can make it challenging to apply findings from animal studies to humans. The tumour co-culture application extends this further, supporting Reduction in early-stage cancer drug screening by narrowing down which candidates are worth carrying into further testing.

Replacement and Reduction of Animal Models in Diffuse Midline Glioma (DMG) Research

The work of Prof. Javad Nazarian, MD — *Professor at the University of Zürich, Head of the DIPG/DMG Research Center at University Children's Hospital Zürich.*

The challenge

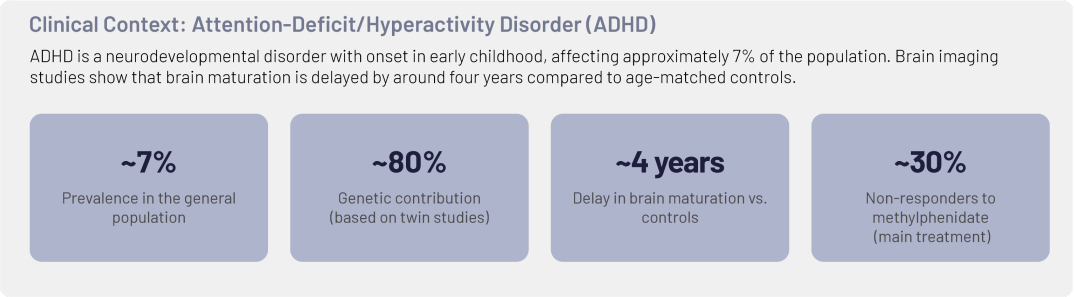
Diffuse Midline Glioma (DMG) is a highly aggressive type of brain tumour mostly affecting children between the ages of 5 and 9. Because the tumour grows in the brainstem (the part of the brain controlling vital functions like breathing and heart rate), surgical removal is not possible.

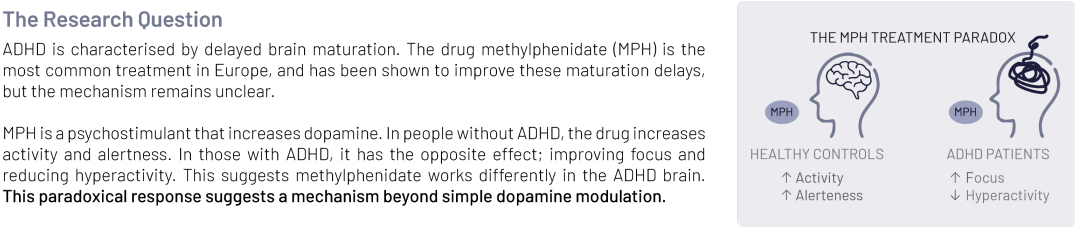
Replacing Patient-Derived Models for ADHD

The work of Prof. Dr. Edna Grünblatt — *Professor at the University of Zurich, Head of Translational Molecular Psychiatry at the Department of Child and Adolescent Psychiatry & Psychotherapy, Psychiatric University Hospital Zurich.*

The Challenge

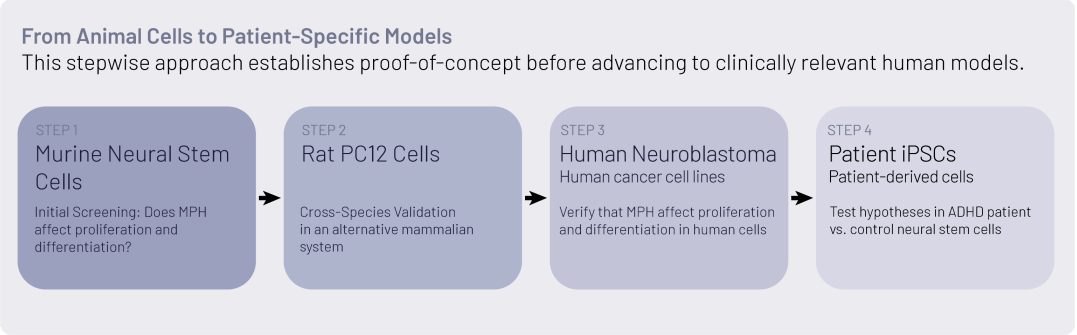
Attention-Deficit/Hyperactivity Disorder (ADHD) affects approximately 7% of the population and is characterised by delayed brain maturation. It is often treated with methylphenidate, the most common treatment in Europe. In people without ADHD, methylphenidate can act as a psychostimulant, increasing activity and alertness. In many people with ADHD, it has the opposite effect on behaviour, improving focus and reducing hyperactivity. This apparent contradiction raises questions about how ADHD brains differ at the cellular level and how methylphenidate acts in that context. To study these questions, researchers need models that reflect human brain development and genetics more closely than is possible in traditional animal experiments alone.



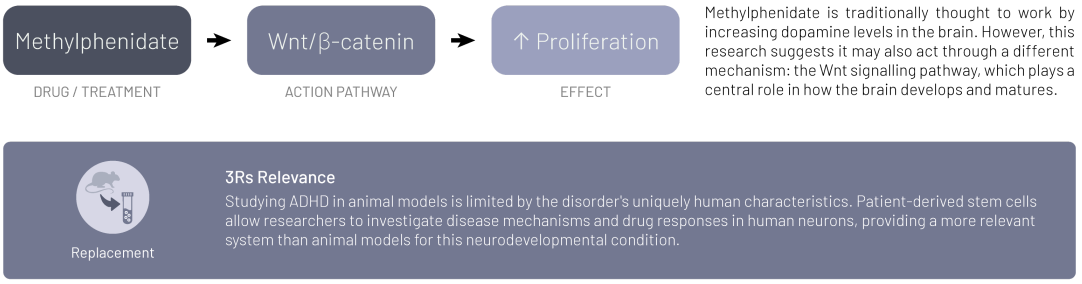


The 3Rs approach

Prof. Grünblatt's group uses induced pluripotent stem cells (iPSCs) taken from patients and healthy controls, reprogrammed in the laboratory to an early developmental state from which they can be grown into specific cell types. From these iPSCs, the team generates patient-specific neural stem cells and cortical neurons (brain cells from the outer layer of the brain). This approach allows scientists to compare brain cells derived from individual patients with ADHD with those from healthy controls, directly in the laboratory.



The team observed changes in the Wnt signalling pathway in ADHD patient cells. A pathway is a series of molecular events that control cell behaviour; in this case, the Wnt signalling pathway plays a central role in how brain cells grow, divide, specialise and form connections.
Preliminary results showed that methylphenidate influences how neuronal stem cells proliferate (divide) and differentiate (mature into neurons). In ADHD patient cells, methylphenidate increased cell proliferation by 18% after just seven days of treatment, compared with untreated ADHD cells, while healthy control cells showed no change. This suggests that the Wnt pathway contributes to how methylphenidate affects ADHD patient cells, although further work is needed to fully clarify the mechanism.



Looking Ahead

These research projects illustrate the ongoing shift in how neuroscience research is being carried out. Human-based non-animal methods are no longer just promising ideas: they are being actively developed and applied to answer important questions about brain health and disease. This progress shows that the 3Rs, as guiding principles, are increasingly being implemented in practice. As these approaches continue to mature, they pave the way for a future of neuroscience that is both more effective at understanding the brain and more humane.

This article is based on research presented at the Swiss 3RCC webinar “Replacement in Neuroscience”, held on 13 November 2025.