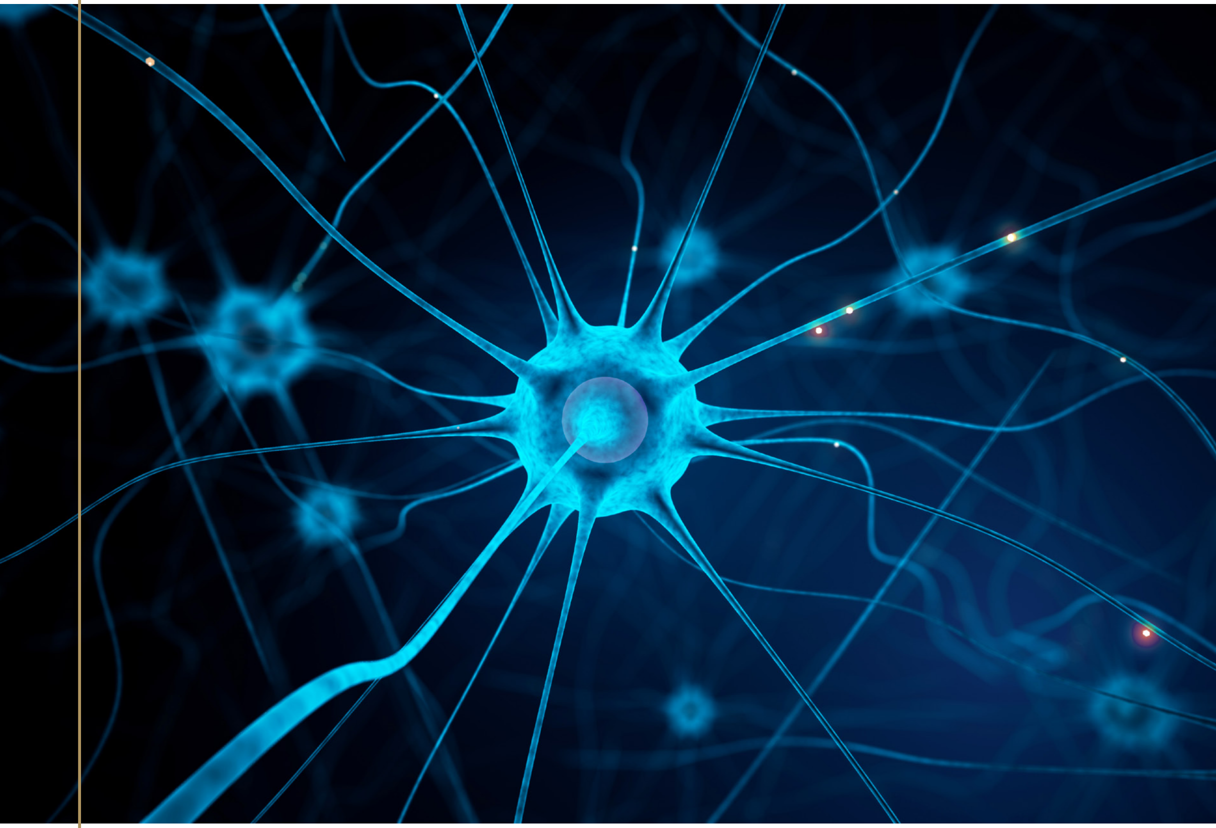
KONINKLIJKE NEDERLANDSE
AKADEMIE VAN WETENSCHAPPEN

INVENTORY

THE IMPORTANCE OF ANIMAL TESTING AND OPPORTUNITIES FOR REDUCING ANIMALS USED IN FUNDAMENTAL NEUROSCIENTIFIC RESEARCH



SUMMARY

In response to a request from the Dutch Ministries of Education, Culture and Science (OCW) and Agriculture, Nature and Food Quality (LNV, previously a part of the Ministry of Economic Affairs), the Royal Netherlands Academy of Arts and Sciences (KNAW) has prepared an inventory of innovative research methods in basic neurosciences that might have potential to boost ongoing efforts to replace, reduce or refine animal experimentation. To that end, developments in a broad range of innovative methods and techniques, ranging from molecular techniques to research methods at the population level, have been described and evaluated.

For the Academy, one key question was whether new techniques or methods will be able to provide answers to the most important current research questions in the neuroscience field. A second precondition was that the quality of research, especially in an area in which Dutch research excels today, should not be impaired.

Basic research

The scope of this inventory is limited to basic research in the neurosciences. For the purpose of this report, the Academy defined the term as all research that seeks fundamental understanding of central nervous systems in changing environments, including research that is inspired by a quest to find useful applications.

In the Netherlands, much basic neuroscience research is indeed driven by a desire to find answers to major challenges our society faces. For example, one in five deaths in the Netherlands is the direct or indirect consequence of a brain-related disorder and healthcare costs for such disorders exceed € 25 billion annually. That requires a better understanding of what has been called the most complex living system in the universe: billions of developing and self-organizing human brain cells and their countless connections with immediate and wider environments.

Basic research is intrinsically unpredictable. In ten years' time, new insights, new research methods and techniques or new research questions may have thoroughly changed the perspective in a scientific field.

Current questions for basic neuroscience research

Basic neurosciences study all aspects of the brain, the central nervous system, the senses and the interrelationships between all these aspects, including their interaction with environmental factors, for example through cognitive and social behaviour.

For this inventory, examples of unanswered research questions were collected. These questions exemplify the central challenge for the discipline: learning to understand the interaction between all biological levels, from molecules and cells to populations and environments. It requires the integration of research at all these levels and a wide range of models, methods and techniques. In addition to in vivo and in vitro research, these also include silico, ex vivo, clinical, genetic, behavioural, epidemiological and ecological research.

Animal experimentation in neuroscience research

Historically, animal experimentation has always been an important element of neuroscience research. Most of the field's body of knowledge has come from research that included such experimentation.

Animal experimentation enabled researchers to do long-term and controlled (behavioural) studies and study several generations over a relatively short period of time.

Powerful or even spectacular innovative techniques in areas such as transgenesis, cellular imaging, electrophysiology and optogenetics can only be used in animal experiments.

Thanks for example to the development of CRISPR-Cas, a technique with which genes of laboratory animals such as mice, rats and fish can be modified very precisely, research can now unravel the contribution of individual genes to complex brain processes and diseases. Specific gene defects that contribute to human diseases can now be reproduced in animal models.

Significant progress has also been made on methods to visualize animal brains and to measure their activity down to the cellular level. Using a technique called optogenetics, researchers can now even use light pulses to switch individual brain cells on and off.

In recent years, these revolutionary techniques and their potential for generating important knowledge have intensified the use of animal experimentation all over the world. In the Netherlands, innovative techniques and better animal models have been invested in as well.

At the same time, great progress has been made in recent decades in reducing animal experimentation as much as possible, in basic neurosciences as well as in other fields. Crucial were the ' 3Rs', which form the basis for policy and regulations in the Netherlands. According to this principle, wherever possible, researchers strive to (1) replace animal experiments with alternative methods that do not require animals, (2) reduce the number of animals per experiment as far as methodologically justified, and (3) refine animal experiments so that animals experience the least possible discomfort. The Academy wholeheartedly supports and applies this 3R policy.

Application of the 3Rs is regulated by law in the Netherlands and monitored through rigorous procedures. Also, for each animal experiment in the Netherlands, the discomfort for animals is ethically weighed against the importance of the knowledge to be acquired. Dutch researchers therefore only use animal experiments if that is necessary to answer important research questions. They also make effective use of suitable alternatives, either those that have recently been developed or those that have been tested over time.

In recent years, a growing number of animal experiments for basic research generally have been registered. Precise numbers for the neurosciences specifically are not being collected. It can however be deduced from granted permits that in recent years the vast majority of animal experiments in the field involved mice, rats and fish.

Innovative techniques and methods

Various recently developed methods and techniques could have potential, at least in theory, to contribute to the 3Rs. This inventory describes five categories of developments:

1. *iPSC technology, organoids and organs-on-chips*: techniques based on tissues derived from human stem cells and miniaturized, microfluidic chip-based devices in which cultured cells interact through tiny reservoirs and channels;
2. *Non-invasive research in humans*; methods that cause no or only short-lasting harm to human subjects, including various forms of imaging, experience sampling through questionnaires and monitoring devices, and testing of human subjects in virtual or augmented reality environments*;*
3. *Reuse of (big) data and tissues*; initiatives aimed at making sets of (raw) data and tissues collected in previous and future research more findable, (freely) accessible, interoperable, and reusable in new research.
4. *Digital modelling:* the use of algorithms and artificial intelligence to model and simulate interactions between various components of the central nervous system in computers;

5. *Invasive research in humans*: techniques for collecting fundamental research data during treatments for certain patients, including deep brain stimulation, transcranial direct current stimulation, and transcranial magnetic stimulation.

Internationally, Dutch researchers have been at the forefront of the development of some of such techniques, such as those related to organoids.

The Academy notes that in the next ten years, innovations in these categories may offer new options for research in which either no, less, or more refined animal experiments are required. However at the same time, the KNAW explicitly notes that these methods and techniques also have serious limitations, and that their contributions to fundamental research therefore currently are and will remain relatively modest.

Conclusions

The KNAW concludes that the innovative methods and techniques described here have the potential to develop further over the next ten years, but that for the time being they will not be able to answer the most important research questions in the scientific field — questions that are crucial for the brain-related challenges that our society faces.

These 'major research questions' require the integration of knowledge and insights at various biological levels, from molecular processes to the development of social behaviour of organisms and populations. They require research with a mix of methods and techniques. In addition to experimental methods that require no or fewer animals, animal experimentation is and will continue to be an indispensable and important basic component of that mix in the foreseeable future, and necessary for doing high-quality fundamental brain research.

The Academy cannot make reliable predictions about the number of animal experiments that will be required in basic neuroscience research ten years from now. While some developments might reduce the need for animal experiments in some instances, other developments will, certainly in the short term, probably increase the need for animal experimentation.

The KNAW points out however that per animal experiment much more knowledge is being collected today than ever before. Basic research in the field is increasingly focusing on mice, rats and fish and less on larger mammals. The Academy expects both trends to continue in the coming years.