Ethical interventions

An expert on the challenges associated with using non-human primate experimental models in research, **Professor Eric Rouiller** discusses new efforts to develop best practice guidelines and recent radical improvements to brain imaging methods

Could you begin by outlining how your work at the Swiss Primate Competence Centre for Research (SPCCR) with non-human primates (NHPs) has developed in recent years?

At SPCCR, we have implemented common procedures between the two sites – University of Fribourg and University of Zurich. We achieved this by training the majority of researchers working with NHPs in Switzerland over a three-day course addressing various facets of this animal model in biomedical research. Several young scientists have now completed their training to become independent researchers, able to work with NHPs in a responsible and ethical manner. From a scientific point of view, SPCCR has pursued research projects aimed at refining methods specific to NHPs, as well as completing studies of mechanisms underlying functional recovery following lesions of the cerebral cortex. These data were compared to previous observations on the mechanisms related to functional recovery from spinal cord lesions, emphasising similarities but also differences between the two models.

Why is SPCCR's work so important for ensuring the integrity of research on NHPs?

The skills required to work with NHPs are rare and specific; skills acquired from working with other animal models, such as rodents, cannot easily be applied. As a consequence, it is important that young scientists attracted by the NHP model undergo specialist training. Trainees attending centres such as SPCCR can capitalise on the long-term expertise that exists among the affiliated scientists. Furthermore, it is crucial to coordinate such training and methodological efforts at a national level so that Swiss scientists have the possibility of conducting research projects on NHPs according to the highest standards.

One of the objectives of SPCCR is to create common guidelines for research involving NHPs in Switzerland. How is this endeavour being addressed?

These guidelines should cover all aspects of research conducted with NHPs in Switzerland. To reach this goal and ensure the quality of training, an official module of competence was submitted and validated by the Swiss veterinary authorities, who are in charge of the survey of biomedical research on animal models. SPCCR expertise was complemented by that of external speakers and tutors from other countries, as well as from other environments such as zoos, and ethological issues were presented and critically discussed. It was an opportunity to evaluate new approaches to adjusting conditions for groups of monkeys in the SPCCR housing facilities.

Can you provide an insight into the challenges associated with the use of imaging to detect disease in NHPs?

Imaging in NHPs has become very useful in order to refine methods, and possibly reduce the number of animals involved in research. Magnetic resonance imaging (MRI) offers high precision by targeting a given brain area subjected to a subsequent restricted lesion, as well as regular monitoring of transformations to the lesion site resulting from reduction of inflammation, oedema, reorganisation of the tissue and so on. If the spatial resolution can be improved further, then imaging data may replace histological assessment to some extent, opening the possibility of prolonging the period of post-lesion observation, which may be useful to test late rehabilitation strategies. In terms of positron emission tomography (PET), a major challenge is to improve resolution and markers in order to trace the fate of implanted cells *in vivo* during functional recovery.

Looking ahead, what is the core goal of SPCCR for the next five to 10 years?

In the future, SPCCR will aim to make our previous experience and controlled NHP models available to the Swiss scientific community, particularly to laboratories focused on translational research, which need to access NHP models in order to validate principles developed in rodents. This will hopefully open up the possibility of conducting clinical trials. However, SPCCR still pursues studies of fundamental mechanisms, as their understanding remains the basis of future therapies. Indeed, basic research and clinical research form a continuum and should not, therefore, be separated.

Setting the standard

The **Swiss Non-Human Primate Competence Centre for Research** is refining primate models used in research in order to deliver high-quality science obtained using robust ethical principles

THE USE OF animals in research is an endlessly contentious area, with academic researchers and institutions, major medical associations, governmental panels and patient organisations stating that most medical breakthroughs in the past hundred years have relied on the use of animals, while others argue that the practice is cruel and scientifically misleading. What is unquestionable, however, is contemporary research's dependence on animals; currently, several million vertebrates are used every year for research.

Despite conflicting views on animal welfare, there is undeniably room for improvement in scientific practice, and a need for greater regulation. Scientists at the Swiss Non-Human Primate Competence Centre for Research (SPCCR) are endeavouring to optimise the use of non-human primates (NHPs) – popular research models because of their genetic similarity to humans. In the past few years, the Centre has continued to make great progress towards achieving the more ethical and effective use of NHPs in scientific research.

GUIDING LIGHT

One of the major goals of the Centre is to establish a set of standards and improved common guidelines for working with NHPs in Switzerland. Since the Centre's establishment, Professors Eric Rouiller, Gregor Rainer (University of Fribourg) and Kevan Martin (University of Zurich) have strived to establish best practices to regulate all aspects of working with NHPs, from animal welfare to surgery methodologies. Two years ago, this was only in discussion, but the Centre has now fully established these unified guiding principles.

METHOD REFINEMENT

While SPCCR adheres to all three 'Rs' of animal welfare – replacement, reduction and refinement – it dedicates particular efforts to refinement. Indeed, much of the work ongoing at the Centre aims to make techniques more animal friendly.

For many experiments, especially those involving brain imaging, it is necessary to restrain the head of the primate. Traditionally, stability has been achieved with a head post anchored using cement. "This may have a detrimental effect on bone and can lead to infection," elaborates Rouiller.

> What unites these models is that they all represent a compromise; the health of the monkeys is sufficiently affected so that they can provide new insights on disease, but without causing unnecessary pain

Laboratories across the globe have been working to find new methods that avoid such issues. Making a valuable contribution, SPPCR recently designed adaptable implants based on a bone-compatible material created from a replicate of the primate skull. "The team at the University of Fribourg has also developed a fixation system based on a helmet adapted to the monkey's head, which doesn't require an implanted head post," Rouiller adds.

A GLOBAL APPROACH

As well as improving conditions for the subjects, SPCCR researchers have been working hard to improve the integrity of results obtained from animal experiments. Electroencephalography (EEG), the recording of electrical activity in the brain, is used in research across multiple disciplines including neuroscience and psychology. While single neuron electrophysiological methods can offer high-resolution data, they are also invasive and generally focus on a small area of the brain. However, because some neurological disorders can impact regions distant to the initial pathogenic lesion, it is important to be able to

THE 3 Rs

The Swiss Non-Human Primate Competence Centre for Research adheres to the three 'Rs' – a set of guiding principles for the ethical use of animals in testing

REPLACEMENT: the preferred use of non-animal methods wherever possible

REDUCTION: methods that enable scientists to obtain the same/comparable levels of information from fewer animals, or more information from the same number of animals

REFINEMENT: methods that minimise the potential pain, suffering or distress experienced by animals

assess neural activity across the entire brain with excellent temporal resolution; EEG is an appropriate method to achieve this, goal.

Members of SPCCR developed a novel, high-density form of EEG. The method, which involves wearing a cap containing 32 electrodes, is currently conducted under anaesthesia, although the team is actively working to develop a next-generation protocol for use on conscious animals. Such a method would enable scientists to monitor the neural activity of the entire brain in relation to different behaviours.

DISEASE MODELLING

Alongside developing techniques, SPCCR has established a number of novel disease models. Its focus is diseases of the central nervous system, and the researchers are renowned for their work in the area of spinal cord injury and lesions in the cerebral cortex.

More recently, Rouiller's group implemented a model for Parkinson's disease, a devastating, degenerative illness that killed over 100,000 people in 2013. Macaque monkeys are treated with a precursor to the neurotoxin MPP+, a chemical that causes the symptoms of Parkinson's disease by destroying dopaminergic neurons. The monkeys were given controlled, consecutive injections of the compound MPTP, until these symptoms appeared. In three out of four monkeys, 80 per cent of their dopaminergic neurons in the substantia nigra were destroyed.

Now, the Centre is working on a treatment for Parkinson's. They are implanting adult neural progenitor cells into the monkeys' striatum, the part of the brain that coordinates movement with motivation, which is seriously affected in patients with the disease. The progenitor cells are obtained from the cortex of the individual into which they are re-implanted. As a result, this approach avoids issues associated with therapies based on stem cells derived from another individual, both in terms of ethics and the need for immunosuppressive treatments. It also means that each individual monkey can be its own control – by comparing motor performance before and after the toxin is administrated.

Although the implanted cells are not themselves dopaminergic, their ability to migrate and establish new connections can recover some lost function. To investigate the human potential of this novel cell therapy, Rouiller and his colleagues are testing the approach further and using imaging methods to understand the mechanisms underlying recovery.

AN ETHICAL TRADE-OFF

What unites these models (Parkinson's, spinal cord and cortical injuries) is that they all represent a compromise; the health of the monkeys is sufficiently affected so that they can provide new insights on disease, but without causing unnecessary pain and discomfort. "The monkeys exhibit the clear symptoms needed for the tests, but not to such a severe extent, so as to preserve an ethically decent health condition," Rouiller reiterates. In the cortical and spinal cord models, this is achieved by inducing lesions in spatially restricted and precisely targeted zones. This means the symptoms are also restricted to a limited region of the body – in most cases just one hand. The same is true of the Parkinson's disease model, in which just enough neurotoxin is administered to induce the necessary level of deficit, and no more.

Animals are imperative to biomedical research – as yet, science does not have the means to conduct essential research without them. The work at SPCCR is therefore an important pursuit; finding new ways of enabling this research to continue, while minimising harm to the animals involved. "Our longstanding experience with such models allows us to ensure the best compromise between effective and ethical goals," he concludes.

SWISS NON-HUMAN PRIMATE COMPETENCE CENTRE FOR RESEARCH

OBJECTIVES

- To coordinate activities between the Universities of Fribourg and Zurich concerning all non-human primate (NHP) related issues
- To foster the exchange of information and sharing of knowledge across the wide range of issues concerning NHP research
- To coordinate R&D of techniques and protocols with a focus on improving the welfare of NHP used in experiments

KEY COLLABORATORS

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PROFESSOR ERIC M ROUILLER gained his BA in Biology and PhD in Physiology from the University of Lausanne, Switzerland, where he then carried out postdoctoral research before moving to the University of Fribourg. Rouiller has held his current position of Full Professor of Neurophysiology at the University of Fribourg since 2003. In addition to his research, Rouiller teaches undergraduate students and supervises PhD students.

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