August 2015 | BioFokus Spezial

BioFokus Spezial

Non human primates as models in research – The view of scientists

www.basel-declaration.org

Forschung für Leben

www.forschung-leben.ch

IMPRESSUM

BioFokus
ISSN 1661-9854
25th volume

Editors
- «Forschung für Leben» («Research for Life»)
  President: Prof Dr Dr Michael Hottiger
  www.forschung-leben.ch
- Basel Declaration Society
  President: Prof Dr Rolf Zeller
  www.basel-declaration.org

Authors
- Prof Dr Rüdiger Behr
  Stem Cell Biology Unit/
  German Primate Center GmbH/
  Leibnitz Institute for Primate Research/Göttingen, Germany
- Prof Dr Gerhard Heldmaier
  University of Marburg, Marburg a.d. Lahn
- Prof Dr Hansjörg Scherberger
  German Primate Center GmbH/
  Leibnitz Institute for Primate Research/Göttingen, Germany
- Prof (em.) Dr Hans-Peter Schreiber
  Bioethics ETH Zurich/Switzerland
- Prof Dr Stefan Treue
  German Primate Center GmbH/
  Leibnitz Institute for Primate Research/Göttingen, Germany

Editorial staff
Prof Dr Felix Ehrensperger
Prof Dr Martin Schwyzer
Astrid Kugler, Managing Director

Layout
Pomcany's Marketing AG, www.pomcanys.ch

Office
- «Forschung für Leben»
  Aargauerstrasse 250
  8048 Zürich
- Basel Declaration Society
  Postfach 33
  4009 Basel

Contents

«In all our ethical rulings and actions in research, we need a responsible and ethical direction that continues to remind us that abolishing research in the name of abstract animal rights legislation would be extremely morally irresponsible in the face of the millions of human beings suffering from dementia today and even more so in the future»

Hans-Peter Schreiber

Editorial

Animal testing makes a vital contribution to the generation of essential biomedical findings, to the development of new drugs, and to new treatment concepts for both humans and animals. Even though a considerable fraction of biomedical research already uses testing methods that do not involve experimenting on animals, a series of questions can only be answered by means of animal testing. Animal testing is used when complex processes and interactions in the living organism must be investigated. There has been controversial and deeply polarising discussion about the use of non-human primates in biomedical research, particularly in the neurosciences, for many years. The internationally published results of these studies, however, make plain that it is precisely research using non-human primates that is and will remain an important component of biomedical research in the 21st century. Above all, the use of primates in basic research has deepened our understanding of neurobiological relationships enormously. Among other things, for example, most of our knowledge about the function of individual areas in the brain comes from experiments on monkeys – this is because important structures in the brain stem, the cerebellum and the cerebrum function in the same specialised way as in humans, only on the evolutionary stage of primates.

Experiments on non-human primates therefore will be of great importance for research into human diseases in the future as well. A circumstance, incidentally, that the revised EU Directive 2010/63/EU on the protection of animals used for scientific purposes, expressly recognises with regard to the use of non-human primates in basic research (Articles 5 and 8).

As regards the ruling of the Swiss Federal Court on primate research at the University of Zürich, handed down in October 2010, it must be noted that biomedical research cannot – as the court decision suggests – be selectively divided up into basic research and applied research. Rather, medical research is a continuum that encompasses both the principle-based basic research of biological processes in healthy as well as sick organisms, and the clinical development and testing of innovative treatments.

The consensus is that all research projects involving animal testing must comprehensively accommodate legislative animal rights and ethical requirements. Above all, this compels both the researcher and the animal testing commissions of the cantons to weigh the competing interests, as required by animal protection law.

From the perspective of a medicine oriented towards innovation and progress, in the future it will be impossible to do without the use of primates in research. Such use, however, will be carried out only in line with a strict weighing of competing interests, and with strong awareness of our great responsibility towards every laboratory animal. In all our ethical rulings and actions in research, we need a responsible and ethical direction that continues to remind us that abolishing research in the name of abstract animal rights legislation would be extremely morally irresponsible in the face of the millions of human beings suffering from dementia today and even more so in the future; all those hoping for both a scientific explanation of their suffering and for the development of new treatment options.

The following contributions from the field of neuroscience make clear that a sharp distinction between basic research and applied research can no longer be maintained, given the fact that most questions to be explained via basic scientific research remain unsolved problems in clinical practice.

Hans-Peter Schreiber

Animal testing: does it make sense?

Rüdiger Behr

As a child I can clearly remember flicking through a magazine and being greeted with the sight of monkeys used in animal experiments. Back then I wondered why people used animals in laboratory tests; why they would subject them to possible pain in the process. Today, I myself do research – including on monkeys.* In my view, in some fields this is the only way to obtain results that will one day benefit modern medicine. I would be very pleased if you would take a few minutes to read on with an impartial mind about the reasons that have brought me to carry out tests on animals. Perhaps you will learn a few new arguments, regardless of whether or not you are convinced by the justifiability and advantages of animal testing.

I will start with a couple of reflections on the weighing of interests between animals and people, a process that also implies a relative evaluation of human and animal life. At this point I would also like to provide a few figures on the „consumption" of animals in our society. I will then move on to illustrating the proven and expected benefits of animal testing by means of three topic areas: organ transplantation, cell replacement therapy and the establishment of new model organisms for research of human diseases. I would like to conclude with a short plea for what is known as basic research – including on animals.

Why am I prepared to risk the lives of animals?

Or better put: Which human interests are more valuable to me than the integrity of animal life?

I can agree with many demands of animal welfare groups. For example, I found the demand for a ban on animal testing in cosmetic research, as is now in force in the EU and Switzerland, very worthy of support. So too is the demand for a ban on distressing and traumatic animal transport, not to mention the ban on the private rearing of many animal species by „animal lovers", as the animals are often not cared for in a species-appropriate way. I also view fur farming with an extremely critical eye. The list could go on. It is therefore not at all my belief that people should have indiscriminate and arbitrary command over animal life.

I strive to protect the grasshopper when mowing the lawn; I don't simply „cut him down" – an anecdotal indication that I am not indifferent to animal life, even the „lower" invertebrate animals. And yet I perform experiments on vertebrate animals. How can that be? When I consider using animals in experiments, I need good reasons that must be weighed against other arguments. In the grasshopper example, the grasshopper's (presumed) interest in life and my interest in mowing the lawn are in

* When we talk about monkeys, we mean Rhesus monkeys (Macaca mulatta), cynomolgus monkeys (Macaca fascicularis) or common marmosets (Callithrix jacchus). These are the most commonly used species in pre-clinical research. Experiments on great apes (chimpanzees, gorillas, orang-utans) are fundamentally forbidden in Europe and no longer carried out here. To prevent another misapprehension: in the work field discussed here, laboratory animals are often only subject to potential pain and suffering in isolated incidents and in the short term. Moreover, relief of pain is achieved by state of the art analgesics. The stress is often caused by procedures that occur similarly in daily veterinary practice – something usually viewed favourably by the population – for example castrations. The animals are kept under the best veterinary care and conditions, something that is also in the interest of the researcher, who naturally wishes to perform his or her studies on animals that are not constrained or burdened by stress. Furthermore, the greatest value is attached to species-appropriate handling, although it must be conceded that laboratory animals sometimes cannot be reared or kept completely species-appropriately i.e. in the sense of recreating their natural habitat. In short: procedures are carried out on animals, but the animals spend most of the time undisturbed, in a manner that satisfies the highest animal handling standards, thus subjecting the animals to as little stress as possible.

** In my view, there are several very useful and reputable ideas and approaches, some tests and experiments carried out today on animals, that should be carried out on cultivated cells in vitro (however, attention must always be paid to where the cells for these tests come from, and whether an animal must be killed to obtain them). As a partial substitute for, and supplement to, tests on living animals, these new approaches appear very promising. However, there are always claims that animal testing could be replaced entirely by other methods and that these replacement methods would provide even better and more meaningful results. I consider these claims false. A mammal is such a complex being (a «system», technically speaking) that only a few isolated aspects and functions of the entire organism can be investigated and analysed on isolated and cultivated (human) cells or even in cell-free biochemical systems. Trying to infer the biochemical, physiological and developmental biological interplay between cells, tissue and organs in the complexity of the entire organism reliably and fully from in vitro cultivated cells or other test systems, is, in my view, an absolutely futile undertaking despite the latest modern computer simulations and miniaturised and automated biotechnology. Those who claim to be able to do so disqualify themselves from a serious discussion of the issue, as they would consequently claim to have understood life itself on all levels (genetic, biochemical, physiological, psychological). Here are four more brief reflections and explanations on this:

a) Unknown components in the system. When I was studying, I learned that there are three types of ribonucleic acid (RNA; transcripts of the genetic material DNA): messenger RNA, transfer RNA and ribosomal RNA. Since then, knowledge about the variety and biological and functional significance of RNAs has increased dramatically. Today, we know about the large group of functionally very diverse small RNAs, long non-coding RNAs, antisense RNAs, etc. All have a great functional importance in cells, and play a role in important processes such as embryonal development and the emergence of cancer. These «new» RNAs are an example of very significant discoveries made in recent years; further examples such as epigenetics could also be mentioned. It would be foolhardy to assume that over the next few years and decades, there would be no more equally significant new discoveries and findings with regard to cell functionality. So how can we carry out meaningful computer simulations of highly complex cellular and molecular biological processes when we are working on an incomplete basis of knowledge? I, at least, fail to understand this.

b) New test systems and their limitations. There are more and more promising miniaturised cell culture systems. These animate test systems equipped with cells on a microscopic scale (lab on a chip) can now mimic individual aspects of a tissue function very well. Likewise, the field of work on so-called organoids continues to grow apace. Organoids are complex three-dimensional cell clusters (tissue) cultured in vitro from (stem) cells, and they replicate specific features of natural organs. Thus for example brain and stomach organoids have been produced. But however complex and similar such structures and systems might be to a natural organ, they are at present – and in my view in the foreseeable future too – unable to replicate the interplay between all relevant organs and tissues in a whole organism, even approximately. Let me be clear: I would find it wonderful if animal testing could be replaced by alternative methods equivalently. I am just convinced that this will not be possible today or in the foreseeable future, if at all. I find it dubious – and also believe that it raises false expectations – to pretend that we could enjoy a similar degree of safety and (medical) progress as we do today if we got rid of animal testing.

c) The complexity of an organism. One of the simplest multicellular animals often used in research is the roundworm Caenorhabditis elegans. Its body always consists of 959 cells in hermaphroditic form (without the germ cells). This is a very clear figure that we can grasp and understand, and also makes this worm highly interesting to biological research (in addition to other criteria). By contrast, the number of cells that make up a typical mammal body is extremely large – so large that it is not at all easy to estimate the number of cells for example in the human body. But the human brain alone consists of about 190 billion cells: written as a figure that's 190,000,000,000. It is a number that in most cases we can hardly grasp. Roughly estimated, an entire human body consists of 100 trillion individual cells (written as a figure: 100,000,000,000,000). Yet it is not the total number of cells alone that makes a complete organism so complex and unfathomable, but rather the variety of different functions that these cells perform, as well as the functional links between different cells in different organs. Thus for example, the complex function of a kidney cannot be researched very meaningfully if isolated in organ culture, as this organ's function – like that of virtually all other organs – strongly depends on other organs and cells that have a regulatory effect on kidney function via nerves and via the messengers transported by blood. It is therefore necessary to connect organs to a functioning neural and circulatory system in order to investigate an organ's overall function. In other words: animal testing is necessary.

d) Psychological phenomena outside animals and humans? We have no scruples about experimenting on cells (although they are living), as cultivated cells are not able to perceive mental phenomena. Isolated cells have – and there is consensus on this – no psyche. The same goes for brain organoids. The limitations of experimental test systems using cultivated cells or organoids are thus revealed: how can the effects of experiments, for example regarding the influence of certain neurological-psychological conditions or cognitive capacities (key word: neurodegenerative diseases), be investigated exclusively on cells or organoids when they do not exhibit these qualities? It is therefore my belief that animal testing remains indispensable in some fields of research for the foreseeable future.

opposition. In this case, both interests – provided I spot the grasshopper in time – need simply be reconciled and accommodated: I can do so by stopping the lawnmower briefly, moving the grasshopper safely aside, and then continuing the job. When using animals in research, however, the interest of animals to live a pain-free, misery-free and species-appropriate life and the fundamental interest of humans to identify and create new possibilities for curing human disease (and animal disease in veterinary medicine!) are sometimes barely reconcilable, indeed even incompatible.** By assessing each individual case and weighing up all the arguments known to me, I am able to come to the personal conclusion that human interests may rank higher than animal interests.

Every reader who at this point categorically rejects animal testing in research, and who perhaps even leads a vegetarian or vegan lifestyle, should ask themselves whether they too are not also endangering animal life with their personal lifestyle and requirements. For example, we should consider that as car drivers or rail passengers we accept the death of hundreds of thousands, probably many millions of vertebrates in Germany alone. According to current figures, more than 200,000 deer,

Rhesus monkeys, German Primate Center, Kurt Fahrner

boar and stags are injured or killed every year in accidents on German roads. The estimated number of unreported cases is three to five times higher. And we must assume that many „only" injured animals ultimately suffer a distressing demise after an accident, as they bleed to death or starve. The number of other vertebrates such as hares, rabbits, mice, marten-like animals and birds is presumably many more million. Moreover, if a female (or among birds, the male too) is killed during their reproductive period, it is highly likely that the as yet dependent offspring will also starve or die of thirst (in a very miserable way). This suffering would never be tolerated within the framework of animal testing, and rightly so. However, these deaths normally occur behind closed doors, so to speak, so this suffering does not appeal to our emotions in the same way – something that does not improve the distressing situation of the affected animals. Our society therefore accepts the million-fold suffering and death of vertebrate animals for reasons of mobility. Who among us has never travelled by car or train? What about the weighing of competing interests in this case? Mobility against the integrity of life in physiologically highly developed animals? Not to mention balancing diet against animal life. Even if the number of vegetarians or vegans should steadily increase, probably by far the biggest proportion of the vertebrates used and killed in Germany will presumably continue to account for meat, milk and egg production over a long period: an almost unbelievable 750 million animals are consumed per year to this end. After mobility and diet comes a third example of the kind of mass killing of vertebrates tolerated or even prescribed by society: Weil's disease is a very rare bacterial infection among humans that in an extreme case can have fatal consequences. The causing bacteria are transmitted especially through the urine of mice, rats, dogs and pigs. As a result, rat, in particular, are controlled intensively and extensively by public authorities. In Hamburg, for example, there is a „rat regulation" that obliges citizens to report and control rats. This usually takes the form of killing them with toxic bait that may cause internal bleeding over several days. Presumably, the animals suffer terribly during this time. According to estimates, up to millions of animals are affected every year. Once again, this situation would be totally unacceptable within the framework of animal testing, but fighting the spread of the very rare Weil's disease is encouraged and even demanded. We should therefore be careful before rashly assuming that animal death cannot be justified by any human need. At present, the fewer than three million animals used in biomedical tests in Germany appear to be rather a modest figure compared to those above.

Yet a person's arguments do not improve by simply pointing at others. So what are the arguments that persuaded me to carry out animal testing, in the context of weighing competing interests?

A prerequisite for recognising the benefits of animal testing is believing that one life is not categorically the same as another and, that an animal or plant species in which life manifests itself is not absolutely worthy of protection, regardless of the species (I personally view human life as a an absolute good that no one may be allowed to harm). In my opinion, however, the protection of animal life can be weighed by looking at the purpose and/or benefit of the action through which its life will be endangered or even ended. Following this line of thinking, I therefore can save the invertebrate grasshopper from my lawnmower and use the monkey as part of my research – in order to achieve a higher goal (i.e. higher than a halfway decent lawn). From my perspective, the protection of animal life is a good thing, but not one that is absolutely worthy of protection. Below are three examples of good reasons for using animals in the interests of humankind.

Organ transplantation

With the first successful organ transplants over 50 years ago, a qualitatively new treatment entered physicians' repertoire of tools in the fight against a wide variety of severe and fatal diseases. When all other options are exhausted, vital organs such as the heart, kidneys, liver, pancreas and lungs can be transplanted. Unfortunately, far too few organs are readily available, so many patients pass away before a donor is found. Nevertheless, hundreds of thousands of people around the world have now been saved from premature death thanks to organ transplantation.

Are you an advocate for organ donation and transplantation? Perhaps you even carry a donor card and feel proud that you could potentially save somebody's life if the worst should happen. In this case, you should also be aware that this wonderful option in the fight against many diseases would not be around had it not been developed, investigated, tested and improved over the course of decades and still today – using animals. Transplant pioneers and Nobel Prize winners Alexis Carrel (Nobel Prize for Medicine and Physiology, 1912), Sir Peter Brian Medawar (Nobel Prize, 1960), Jean Dausset (Nobel Prize, 1980) and Joseph Edward Murray (Nobel Prize, 1990) used dogs, cats and rabbits for their trailblazing work on organ transplantation. And the pioneer of heart transplants, Christiaan Barnard, performed studies on baboons and rats. Even today, experimental transplantation studies are carried out on animals, including monkeys, in order to con-

Monkey compound, German Primate Center, Kurt Fahrner

tinue improving medical procedures and thus patients' chances of survival. Since 1963, more than 100,000 heart, kidney, liver, lung and pancreas transplants have been performed in Germany alone. However, it is depressing to note that the demand is unfortunately much greater; around 11,000 transplants are currently needed in Germany alone to treat all the patients on the waiting lists. And many patients pass away before a donor organ can be found for them. Every single transplant is therefore extremely valuable, and decisions must be made regarding which of the many fatally sick people will receive the privilege of a donor organ and which patients with an acutely high risk of death cannot be provided for. In this context, ethical considerations compel the use of the best available transplant procedures – and improvements in transplant procedures must be sought to continue boosting success rates. In some cases, this can – and I believe this is plausible – only happen with experiments on animals. Those who do not rank the life of humans and animals the same, and do not fundamentally or categorically allow animals the same protection status as people, should be able to see a huge benefit in animal testing here. Those who value all life the same and reject the use (and also killing) of animals for the preservation of human life, however, should explain to the patient waiting for a donor organ that the treatment s/he is hoping for is unethical, as its development is based on the use and killing of animals.

New treatment options through stem cells?

Stem cell therapy could become another important innovation in treatment over the next few years and decades. If the concept paves its way into broad clinical use, it will likely have a far greater impact on human medicine than did organ transplantation. Embryonic stem cells (ESC) are all-rounders. They are isolated from a very early embryonic stage. In this stage, the embryo cells are not yet specialised in certain functions; they are undifferentiated. However, they have the potential to develop into any specialised cells in the adult body, for example heart muscle cells, nerve cells, blood cells or liver cells. This capability is called pluripotency. It is not only the cells of the intact early embryo that exhibit this

pluripotency, but also the ESC isolated from early embryos then kept in cell culture. You can therefore obtain specialised cells such as heart muscle, nerve or blood cells from non-specialised ESCs in cell culture, i.e., outside a living organism (in vitro). For a few years now, it has been possible with the help of molecular biological tricks to obtain cells from skin or blood that are very similar to ESC – and no embryos are needed to obtain them. These so-called induced pluripotent stem cells are like the ESC all-rounders. Current research into pluripotent stem cells is largely targeted at cell replacement therapy, that is, the aim is to ease or even cure diseases linked to cell degeneration or cell loss with the aid of pluripotent cells. Research is focussing on Type I diabetes, for example, in which the insulin-producing cells of the pancreas are destroyed; on cardiac infarction, in which the heart muscle cells collapse due to a lack of oxygen; or on Parkinson‘s, in which certain nerve cells in the brain stem degenerate. The essentially simple idea here is to replace the specialised cells lost (degenerated) over the course of the disease in vitro with cells from pluripotent stem cells. Diabetes has already been successfully treated for a limited period of time in mice, for example. The work to date has been tantalising and promising, but there is still a long way to go before routine clinical use becomes possible, as there are still problems to solve. Thus at present work is being done on strategies to minimise the risk of the replacement cells originating from pluripotent stem cells forming tumours in the recipient (patient). Another problem – that varies greatly depending on the disease – also lies in the administration and proper insertion of the new cells into the cell group (tissue) that already exists in the body. How do I put the cells where I need them, and how do I ensure that I insert them optimally into the tissues at the site of the disease? Furthermore, the issue of immune defence also deserves consideration. It is known from transplantation medicine that transplanted organs are attacked and rejected by the recipient‘s immune system, as they are identified as „foreign“. Unfortunately, this applies equally to replacement cells derived from pluripotent stem cells introduced into the body as part of a cell replacement treatment – presumably including the induced pluripotent stem cells, although these cells in theory can be produced for each patient specifically. All these questions and problems on the path to using cell replacement therapy on people can ultimately only be answered in pre-clinical tests. Here too, the same holds true: these tests can only be as good and as meaningful as the „test system“ used. In this case, monkeys are the best species for many issues posed in human medicine, because research on them produces the most revealing and significant results. Before employing a particular stem cell therapy on a patient, it must be tested for effectiveness and safety on animals, ideally monkeys from a natural science point of view. In brief, there are three reasons for this: 1) Pluripotent stem cells in monkeys and humans are considerably different to those of mice. So those, who wish to learn something about human stem cells may probably be misled by working with mouse stem cells. 2) The immune system of primates, the group to which monkeys and humans both belong, is clearly different to that of mice. So those who wish to learn something about the rejection reaction of transplanted cells in patients must depend on animal testing with monkeys. 3) It can take several years before the tumours triggered by stem cells may emerge and can be diagnosed. In order to monitor these periods of time and thus be able to test the long-term safety of a procedure, the animal must also have a correspondingly long life span. Mice have a life expectancy of two years at most; monkeys on the other hand live far longer than ten years.

In discussing such works, it must be clear to the reader that researchers do not carry out experiments randomly or indiscriminately. All studies are carefully planned before starting, and application is made to the relevant authority. When examining these applications, the authority is advised by a panel to which animal welfare representatives also belong. The experiment is only carried out after receiving authorisation, and is subsequently monitored by vets and/or animal welfare officers. In the case of experimental studies on cell replacement therapy, the animals would be painlessly put to sleep once the test period is over in order to investigate the effects of the pre-clinical treatment approaches on an organismic, cellular and molecular level as well. In this scenario too, therefore, we must weigh the expected suffering of the animal (as well as its being put to sleep) against the prospective benefits. Upon weighing all the recognisable advantages and disadvantages, my personal evaluation of the situation is that pre-clinical studies on monkeys are justifiable in order to check the safety and effectiveness of cell replacement therapies, as these experiments may possibly ease the suffering of many people with a serious disease – and ideally even cure them. At this point I would like to state plainly that I do not believe that anybody can today guarantee that in the future patients will be routinely cured with stem cell therapy. However, there is also fundamentally nothing to prevent this form of therapy, still in an experimental stage, from becoming a breakthrough in the treatment of a range of serious diseases. There are useful solution approaches for all known problems. What is lacking, in my opinion, are meaningful pre-clinical studies in animal models that ultimately offer the experi-

mental evidence that a treatment is wholly safe and effective.

New model organisms for research into human disease

I completed my alternative service in lieu of military service in patient care in a ward for geriatric psychiatry patients; I cared for people suffering from severe dementia and depression, bed-ridden for years, and I saw them die. The suffering that I witnessed, for example in Alzheimer's patients and their loved ones, was a deeply formative experience.

Alzheimer's disease is a slow, but inexorably progressing type of dementia typically seen in older people. It usually leads to death six to ten years after diagnosis, and the relatives suffer just as much as the patient themselves. According to current estimates, around 1.2 million people are affected by Alzheimer's in Germany alone. Due to increasing life expectancy, more and more people will suffer from Alzheimer's in future. In addition to the individual fate of patients, the disease also represents a huge economic and health challenge for the country, as the population pyramid becomes less triangular and more like a stretched balloon (meaning that there are fewer people in the younger age groups, who must support the larger numbers of people in the older and oldest age groups). Ultimately, however, looking simply at the individual fate of such patients and their relatives, I believe that doctors, researchers and society as a whole have a great responsibility to treat these patients as well as possible. Animal testing is necessary, among other things, and on a species that is capable of complex cognitive performance and has a long enough life expectancy for researchers to observe the course of a disease over several years, with all its various facets. For neurological diseases including Alzheimer's, Parkinson's or Huntington's, primates are therefore very illuminating laboratory animals. A method by which individual isolated and disease-causing human genes can be introduced into animal genomes could play a bigger role here in future, as could the method of switching off certain genes responsible for the prevention of the emergence of disease. For example, the genes that trigger Parkinson's or Huntington's in humans could be added to the monkey genome – we would assume from this that the monkeys would also develop the disease. Or if an important gene for normal mental development is switched off that, if non-functional, leads to severe brain disorders such as Rett syndrome. While we cannot access the sick cells and tissue in humans (who would agree to tissue removal, particularly from the brain of living patients? – quite rightly, nobody!), this option is both feasible and possible in animals after putting them to sleep. It must be made clear that no patient and no person is to be genetically modified in a way that his children will inherit the same genetic modification. The sole purpose of adding additional genes to monkey genomes is to create new model organisms through which the emergence and possibly also the experimental treatment of disease can be better investigated and understood. I am aware that the thought of adding genes to a monkey, or deliberately making a gene inoperable in monkeys, triggers great unease and repugnance in many people. Yet such animals that already exist in the USA, Japan and China are not "monsters", per se. A neutral observer would presumably be unable to tell the difference between these animals and their unmodified siblings.

In view of all the possible reservations towards the genetic modification of animals in general and of monkeys in particular, we should also always ask the question: is the responsible use and killing of animals including monkeys unethical if by doing so I am pursuing a possible path to better understanding of serious disease among people? Or conversely: would unwillingness to pursue a possible, albeit very long, path to patient treatment be unethical? Is even refraining from work on meaningful model organisms also unethical? I have asked myself this question and weighed the arguments. Certainly because I have been strongly influenced by my experiences caring for dying and suffering patients, I came to the conclusion that the use of animals, including monkeys, is justified for the research of serious diseases. What do you think?

What we call basic research – why is it so important?

Recently, researchers who perform tests on animals have been increasingly asked how long it takes before their work might provide a specific benefit for patients. Time frames of three to ten years are talked about. Demanding a clinically usable „output" of animal testing in such a short time is, however, counterproductive. Moreover, it seems nonsensitive to me to separate what is known as pure basic research (viewed by many people as „evil" i.e. not justified with relation to animal testing) from applied (pre-clinical and therefore „good") research, as findings and ideas for clinical applications often only develop from „basic experiments". The transplant pioneer Alexis Carrel was awarded the Nobel Prize for Medicine and Physiology in 1912 for fundamental work that he had performed

on dogs and cats in the preceding years. However, it took more than 50 more years before organ transplantation was successfully clinically applied: that's about 60 years from the initial scientific work on animals to clinical application. Is it not odd, even unrealistic, then, to demand that research work on animals be ready for clinical application within just a few years?

Another example is stem cell research. Today we are discussing how to pave the way for cell replacement therapy in clinical practice. Today! Work on pluripotent stem cells began in the 1950s and early 1960s, when testicular tumours (so-called teratomas) were observed in a mouse strain; these tumours shared many characteristics of cells from early embryos. Today, this research would certainly be labelled „pure basic research". Almost 35 years ago, the first „true" embryonal stem cells from mice were described. And it has been 20 and 17 years respectively since the first embryonal stem cells of monkeys and man were published. In 2009, three researchers from the USA and England were awarded for their pioneering discoveries in the field of embryonal stem cells. And the first clinical tests to verify the safety and effectiveness of therapies using embryonal stem cells from humans have only begun in the last few years. Again, there is still a very long way to go before a stem cell-based treatment could be more broadly in use among patients.

In terms of great innovations, therefore, several decades may pass between the initial basic scientific work on an issue to clinical application. So if the approval of animal testing in the field of basic research is very restrictive today, the consequences will only be felt in several decades' time. Those who do not wish to let the pipeline of medical innovation run dry – in many fields, at least – should think hard about whether basic research is not actually very useful to people, in a meaningful and long term way. In any case, however, the statement that „The preservation or protection of human life and health is weightier than results about fundamental life processes" (as stated in a ruling prohibiting a specific monkey experiment by the Swiss Federal Court) demonstrates little understanding of how great breakthroughs emerge in medical research.

From animal physiology to human health

Between medical challenges, ethical and legal obligations, and unobjective criticism: why animal studies in basic biomedical research are indispensable

Gerhard Heldmaier and Stefan Treue

The daily reports during the past months on the suffering of Ebola patients and their relatives demonstrate just how helpless science, medicine and politics are in the face of this epidemic: vaccines are tested but are as yet unavailable; specific Ebola drugs to treat acute patients do not exist. They do not exist because we lack basic knowledge. We don't know how these viruses penetrate cells, how they annihilate and outmanoeuvre our body's own defences, or which treatment can be employed to destroy them. Entirely new paths of treatment must possibly be found. A treatment involving fragments of genetic material (siRNA) recently successfully cured Rhesus monkeys infected with the related Marburg virus. However, many additional studies on animals and ultimately also on people are required before a successful treatment becomes available.

Medicine is further advanced when it comes to supporting those people suffering from Parkinson's Disease. Deep brain stimulation enables thousands of patients to avoid the movement disorders that are characteristic of this disease. In this treatment, patients are implanted with an electrode deep in the brain, used to stimulate the regions that in Parkinson's have lost their capacity to function normally. This treatment helps to establish a previously unrivalled quality of life for those affected. In this case too, it was basic biomedical research, including studies and a series of tests on monkeys, that laid the foundations for this treatment.

These two current medical challenges demonstrate that research on animals is necessary – and why. Whenever research focuses on functions in the intact organism, studies on animals are necessary, for example in infectious biology, cardio-vascular research, endocrinology and the neurosciences. Overall, research on animals plays only a minor role in the biosciences, numerically speaking. Instead, cell or tissue cultures are ordinarily used to discover information about biochemical processes, signal transfer or gene expression. But animal research has a key function, in that it is the only way to elucidate the significance of these details for the body as a whole.

Animal research is unavoidable and essential for understanding the foundations of life and to achieve advances in medicine. We impair or destroy life in order to learn more about it – a classic ethical dilemma. This dilemma is heightened by the fact that research also has an ethical responsibility towards people to improve medical care with new findings.

This ethical area of conflict is a big hurdle for all those working with animals in science. Nobody performs animal studies without reason. It is a prerequisite to first have a profound scientific question that promises a significant amount of information to be gained, and that can only be answered through research on animals. Of course, the relevant training and professional experience must also guarantee that the animals come under as little strain as possible. These are essential conditions for the success of a research project, and adhering to them is therefore also in the researcher's own interest.

The 3 Rs – "Reduce, Refine, Replace" – formulated by Russell and Burch in their book "The Principles of Human Experimental Technique" in 1959, now form the guiding ethical principle of research around the world. They oblige researchers to reduce the number of animal experiments to the minimum necessary to answer a scientific question, to refine research methods to minimize the animals' strain, and to use replacement methods for research on animals whenever possible.

Added to the ethical dimension is the legal question – over the last few decades, animal protection laws in Germany have been tightened several times. Among the laws now in force across Europe, there is an official approval process in which representatives from animal protection associations also participate. Experiments can only be approved when there are no alternative methods, and when they serve important goals. If approved, protocol must be followed to the letter regarding the animals used. Veterinary inspection officers and animal welfare officers monitor the animals' keeping, the ongoing projects and make sure that protocol is followed. When the project is concluded, a final report must be submitted to the

approval authority. There is no other branch of research with this level of bureaucratic effort and such a dense control network.

Yet despite the central importance of animal research for medical and scientific progress, and despite all the high ethical (self-)obligations and legal requirements, studies on animals are often critically viewed or even categorically opposed. False claims are sometimes made to argue against any possible necessity or value of animal research; scientists are defamed and threatened.

One of the most common arguments against animal research is the claim that it is unnecessary because animal-free replacement methods are available. This is simply incorrect because studies in animals are only permissible when there are no replacement methods. Replacement methods using cell cultures, for example, are continually being improved and redeveloped. There has recently been success combining several cell types and generating spatial structures resembling tissue. Replacement methods, however, have system-based limits, as these artificially cultivated cells and tissues cannot completely reproduce conditions in the intact animal. Therefore, the findings gained from these artificial systems must ultimately be verified in the intact organism.

Another frequent argument against animal research is the alleged impossibility of transferring findings obtained from animals to humans. Of course, there is no one-to-one transferability from animals to humans – humans are not mice. Due to the similarity of cell and organ function in mammals, however, it is to be expected that it is fundamentally possible to transfer the principles to humans. Nevertheless, careful consideration and an in-depth knowledge of comparative physiology are required before drawing conclusions for humans from the results of studies on animals.

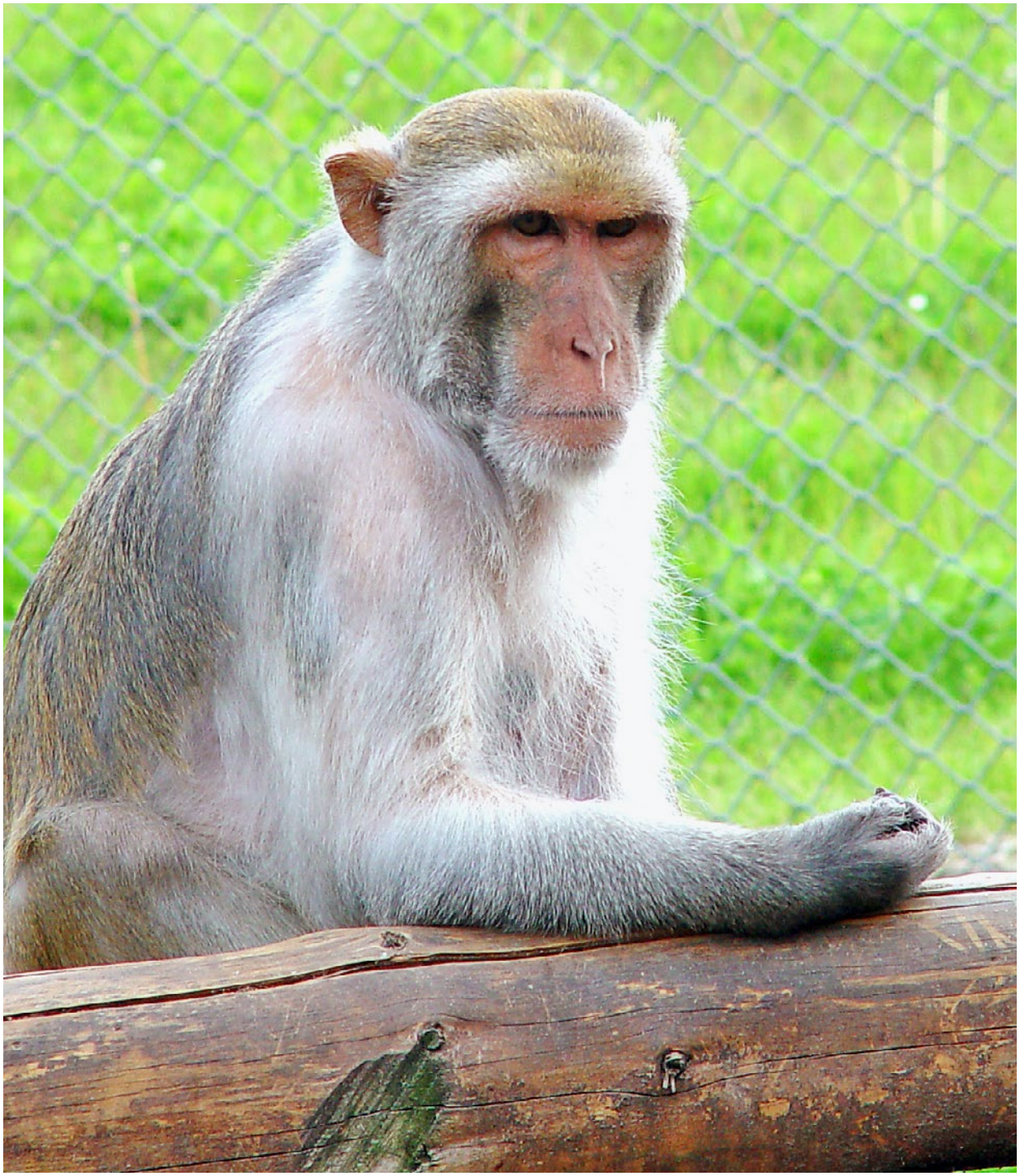
In the discussion about transferability, it is often suggested that the apparently ethically superior applied research can be separated from the allegedly senseless and therefore indefensible basic research. Ultimately, however, the latter is the driving motor for innovation and a prerequisite for all applications. New knowledge about Nature opens up new paths to us – to help us make better use of the natural resources at our disposal and to improve the medical care of humans and animals. Basic research and applied research are therefore inseparable, and subject to the same ethical challenges.

A final argument from opponents to research on animals is that far too many animals are used in experiments. In fact, at present around three million animals per year are used for scientific purposes in Germany. Contrary to what is often suggested, the proportion of primates is extremely small. Despite the great scientific significance of research on monkeys, they make up only 0.05% of all laboratory animals. Almost three-quarters of the animals used are mice (73 per cent), followed by rats (14 per cent). Half of these animals are used for studies, i.e. the animals are treated, for example, with new drugs and the effect is monitored over days or weeks. The rest are euthanized without undergoing prior procedures in order to obtain tissue for research and to apply replacement methods.

At first glance, three million is a shockingly high figure. According to statistics from the Federal Ministry of Agriculture, however, a total of 760 million animals are used at the same time, mostly for our food. This high figure for annual animal consumption in Germany shows that using animals is accepted in our society. We do not only use them for food; we also kill them as pests, we hunt, we fish, we destroy habitats for agriculture, roads and settlements, we keep them as pets and we use them in research. The number of animals used in research amounts to 0.4 per cent of the officially recorded animal consumption. Is that excessive or reasonable for obtaining scientific knowledge and improving our medical care? There is no catch-all answer for this. Each person must decide for themselves whether they accept this situation or not, and if not, then at least think about it the next time they visit the doctor.

In any case, the number of animals used in research will continue to rise in future despite all efforts to find replacement methods – and despite all criticism. Our improved understanding of the similarities and differences between animals and people and the rapidly progressing development of genetically engineered methods enable us to gain new insights into the foundations of life processes and diseases. This gives us hope for new and better diagnoses and treatments; personalised medicine is a buzzword here, that is, treatments tailored especially to a particular patient.

In this context, scientists, their associations and the pharmaceutical industry must educate the public much more openly and clearly about the questions, methods and results of this type of research. Indeed, numerous reports have been published on the importance of animal research and many researchers studying animals have, over the past few years, arranged open-house days at their institutes or spoken about animal research at events. These sources of information meet with great interest from the public, but reach too few people. The majority of



Rhesus monkey, German Primate Center, Kurt Fahrner

researchers have been somewhat reticent in the past, either from a fear of hostility or because they have underestimated the importance of informing the public. In view of the social significance of the topic, this reticence needs to end.

Prof. Dr. Gerhard Heldmaier is an animal physiologist at the University of Marburg and Chairman of the Senate Commission on Animal Protection and Experimentation at the German Research Association (Deutsche Forschungsgesellschaft (DFG)). Prof. Dr. Stefan Treue is a neuroscientist at the University of Göttingen and Director of the German Primate Center – Leibniz Institute for Primate Research as well as a member of the DFG Senate Commission.

Published in FAZ (Frankfurter Allgemeine Zeitung), 22nd October 2014

Neuroprosthetics

Hansjörg Scherberger

Introduction

A patient sits in front of a screen and moves a cursor. He clicks on a control panel, opens an email browser and writes a text. Nothing special? – Except that patient MN does it without moving his hands, just with the power of his mind! Since his neck injury, patient MN has been paralyzed from his neck downward. He sits in a wheelchair, unable to move his arms and legs, and needs a ventilator for breathing assistance. His movement intentions are recognized by means of implanted electrodes in his brain and sent to a computer. These electrodes detect neural activity from single nerve cells. Without these electrodes, his movement intentions could not be recognized. They register continuously the neural activity patterns in specialized brain areas for hand movements, which are subsequently interpreted by a computer, and used as control signals to move a cursor on a screen or to type letters (Figure 1). For patient MN, this task is not effortless, but he can talk to the experimenter while performing. For him, language and this neuroprosthesis are the only remaining channels of communication.

Cases like patient MN are not that rare, unfortunately. In younger patients, paraplegia is often caused by injuries of the spinal cord following accidents at home, during traffic, sports, or at work. Furthermore, the motor system can be affected by several debilitating diseases (e.g., patients with multiple sclerosis or ALS) and by stroke lesions to specific cortical motor areas or descending pathways. These injuries often lead to paralysis of speech or one body hemisphere. Finally, there is a large group of patients with limb amputations, especially after times of war.

Patients with missing limbs can be fitted with prostheses that offer both cosmetic and functional replacements (Figure 2A). Unfortunately, complete functional replacement, e.g., for a missing hand, is currently not possible. This lack is not due to a technical incapacity to build prosthesis with adequate dexterity and motor detail (Figure 2), but to the problem of making them controllable by the patient. This is where neuroprosthetics come into play. The hope is that this technology can help not only patients with amputations but also other patients with severe paralysis or debilitating motor diseases.

What are neuroprostheses?

Patient MN has an intact brain. He can recognize the environment with his senses and process it, but he is severely hindered to interact with it. MN is well capable to develop and formulate movement intentions in the brain, but the resulting control signals are not propagated to the executing muscles because of nerve interruptions in the spinal cord from the injury. Neuroprostheses aim to circumvent, or bridge, these interruptions (Figure 3). In other words, neuroprostheses aim to read out neuronal signals directly from the brain in order to control external devices like a robot hand or a cursor on a computer screen.

Whereas robotics is well capable of building and moving an anthropomorphic robotic hand (Figure 2B), the main problem of neuroprosthetics is, as expected, to read out and interpret adequate control signals from the brain. Experiments with patient MN and others have shown that this is possible. But how?

What kinds of neuroprostheses exist?

From a technical point of view, neuroprostheses provide an interface for information exchange between the nervous system and a computer or technical device. Synonymous names are ‘brain-computer interface’, ‘brain-machine interface’, or ‘neuronal interface’. Common to all neuroprostheses is that they detect signals from the nervous system and analyze them to predict movement intentions in real-time. In order to achieve this, two important points about neural signal selection need to be considered. First, the registered signals should contain information about the intended movement; it is not helpful to use signals that don’t contain the wanted information or with unknown coding scheme. Second, the neuronal signals should be well accessible. Here, external recording techniques are advantageous over invasive methods that record the signals with implanted electrodes (Figure 4).

Noninvasive Neuroprostheses

Non-invasive neuroprostheses are mainly based on electro-encephalogram (EEG) signals. They are recorded with electrodes that are placed on the skin of the skull (Figure 4) and they measure the electric field of the brain spatially smoothed from outside of the skull. The EEG has a very fine temporal, but

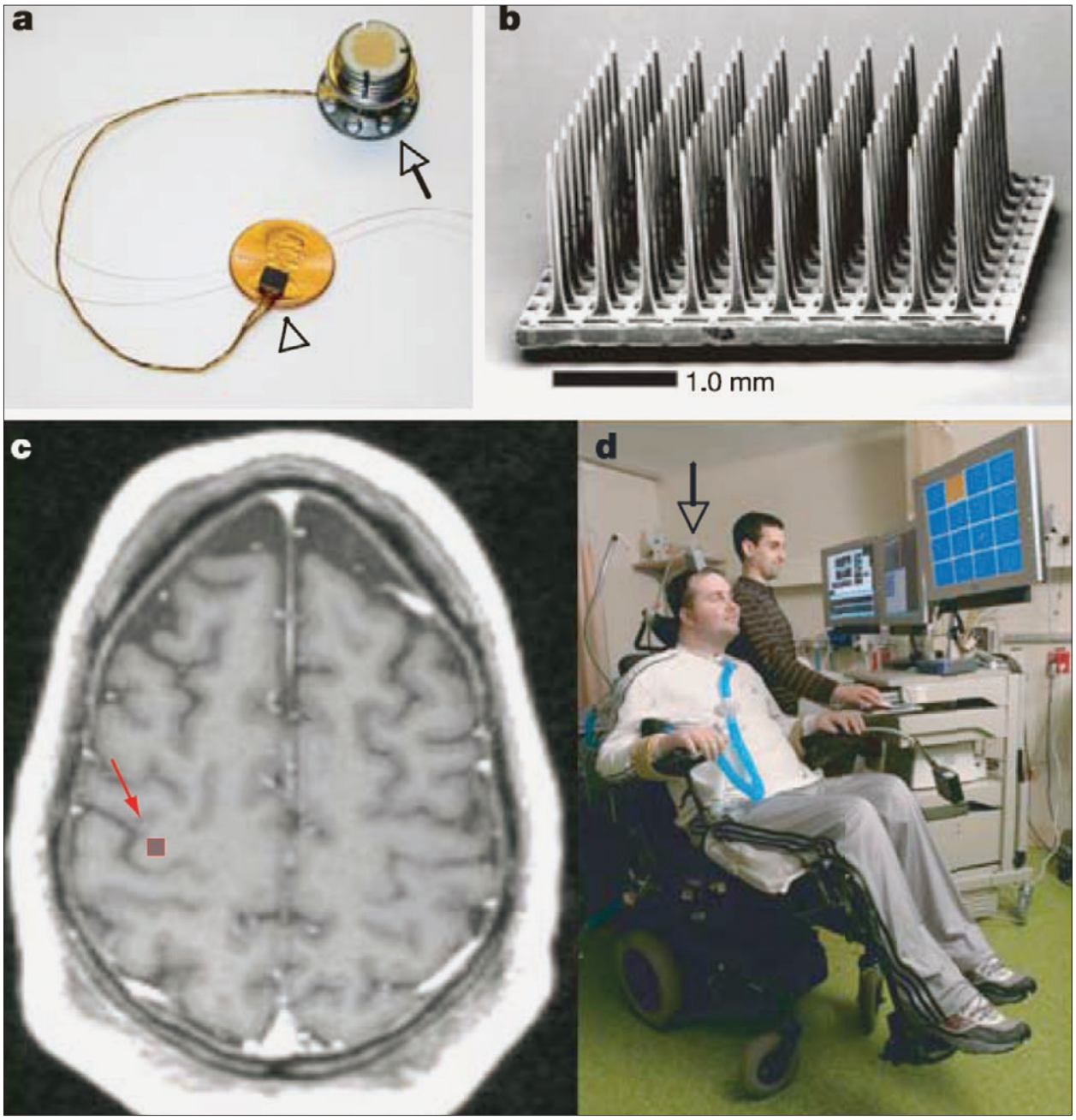


Figure 1: Patient with intracortical neuroprosthesis.
A: *Electrode array (black square on-top of coin) with cable and connector.* ***B:*** *Close-up of electrode array with 100 (10x10) electrodes.* ***C:*** *Location of electrode array in motor cortex of a patient (red array).* ***D:*** *Patient MN (sitting) during an experiment; Neuroprosthesis is connected to a decoding computer (arrow). The patient looks at a blue monitor and moves the cursor to the orange field (Reproduced with permission from Macmillan Publishers Ltd: Hochberg et al., 2006, Nature 442: 164–171).*

only coarse spatial resolution. Functionally, every electrode averages the electrical activity of several square centimeters of cortical (brain) surface. For this reason, the EEG does not show a single, individual movement intention, but rather a summation signal of many simultaneous neuronal activities that are not separable due to the long distance from its origin and the spatial low-pass filter properties of the bony skull (Figure 4).

Nevertheless, many human subjects are capable of voluntarily influencing certain components of the EEG after sufficient training. These signal components can then be used for non-invasive neuroprosthesis control, e.g., for selecting keys on a keyboard or the cursor position on a computer screen.

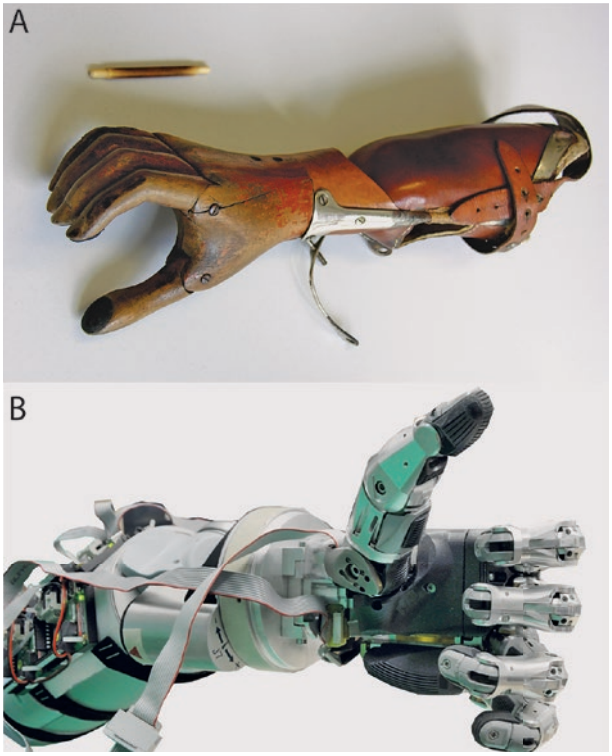


Figure 2: Hand prostheses of yesterday and tomorrow. ***A:*** *Wooden arm- und hand prosthesis after F. Sauerbruch und A. Stodola at about 1918. A simple grasping function was implemented with a sling-mechanism that transmits force from a pierced muscle in the arm stump on the prosthetic hand (Reproduced with permission of the Medizinhistorisches Museum der Charité, Berlin).* ***B:*** *Anthropomorphic robot hand, about 2011. Hand and finger joints are driven by electical motors and have many degrees of freedom.*

Unfortunately, there are several disadvantages that limit the usefulness of such EEG-based neuroprostheses. First, information throughput is limited, which makes control of complex movements difficult, like individual finger movements. Second, non-invasive neuroprostheses are very sensitive to external influences, in particular to visual and acoustic disturbances. Both issues strongly limit the applicability of such systems. Finally, only a fraction of all experimental subjects seem to be able to learn this method. For these reasons, neuroprostheses with implantable electrodes seem to be a viable alternative for many patients.

Implantable Neuroprostheses

For implantable neuroprostheses the sensors (electrodes) are placed either directly in the brain or just on top of it (Figure 4). This offers the possibility to record the activity of an individual nerve cell or a small group of such cells on one electrode, and with an array of such electrodes a large number of such signals can be acquired simultaneously from a single brain area in high resolution. In other words, the local activity of a specific brain area can be selectively monitored.

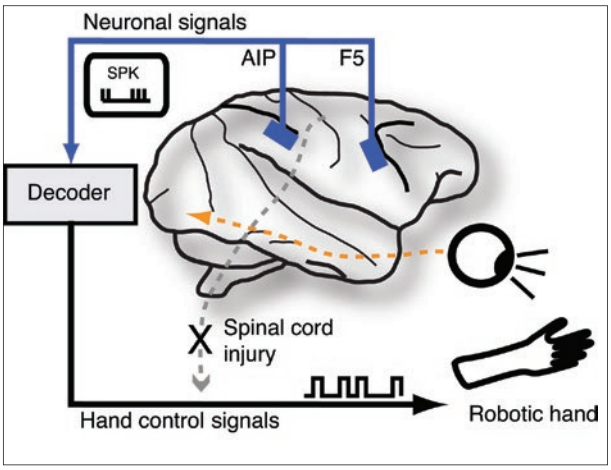


Figure 3: Functional schematic of a neuroprosthesis. Paralyzed patients, e.g. after spinal cord injury, can still perceive the outside world, e.g., by vision (orange dashed arrow), but they are unable to execute the resulting movement intentions, because nerve fibers sending control signals to the motor nerves are disconnected (black X) at the level of the spinal cord. Neuroprostheses record neuronal information (SPK) from specific cortical areas (here from the parietal area AIP and the premotor area F5), from which a decoder predicts the intended movements and propagates them to a robot hand. Finally, the brain can check the executed movement by visual feedback.

For example, hand and finger movements can be decoded from a cortical area that is specialized for the planning and execution of grasping movements, Using implanted electrodes, this can be achieved much more precisely, detailed, and robustly than possible with non-invasive methods (Figure 5). Furthermore, since the recorded signals represent just the local cortical activity and not an average of larger brain areas, they are less prone to external disturbances, like distracting

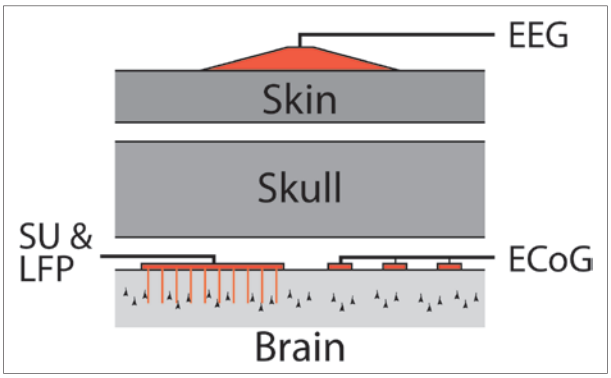


Figure 4: Localization of external and various implanted electrodes for neuroprostheses. ***EEG:*** *Electro-encephalographic (EEG) electrodes are placed temporarily on the skin and record electrical brain signals after traveling through the skin and the skull that strongly average these signals spatially.* ***SU & LFP:*** *Permanently implanted electrode arrays can record single-unit (SU) activity from individual nerve cells and the local field potential (LFP). Electrode tips are placed in cortical grey matter, immediately next to neurons.* ***ECoG:*** *Electrodes for recording of the electro-corticogram (ECoG) are placed onto the cortical surface. They can record local changes of the cortical electrical field, but normally no high-quality SU or LFP signals.*

sensory stimuli, or the simultaneous planning and execution of other movements. This strong specificity of the signals, up to isolated single-cell activity, allows the development of neuroprostheses with high precision and robustness, and with an information transfer rate capacity that significantly exceeds non-invasive methods.

To improve the effectiveness of neuroprostheses, the natural plasticity of the brain can also be exploited. The brain continuously adjusts itself to the ever-changing input and output signals. Therefore, somewhat simplified, the brain can adapt its coding to an erroneous or imperfect neuroprosthesis in order to improve the decoding performance of the brain-machine interface. This is possible if the brain receives sensory feedback about the decoding errors, e.g., when the subject sees the decoded movements. Ultimately, the brain can adapt even to a completely uncalibrated decoding algorithm within days or weeks,

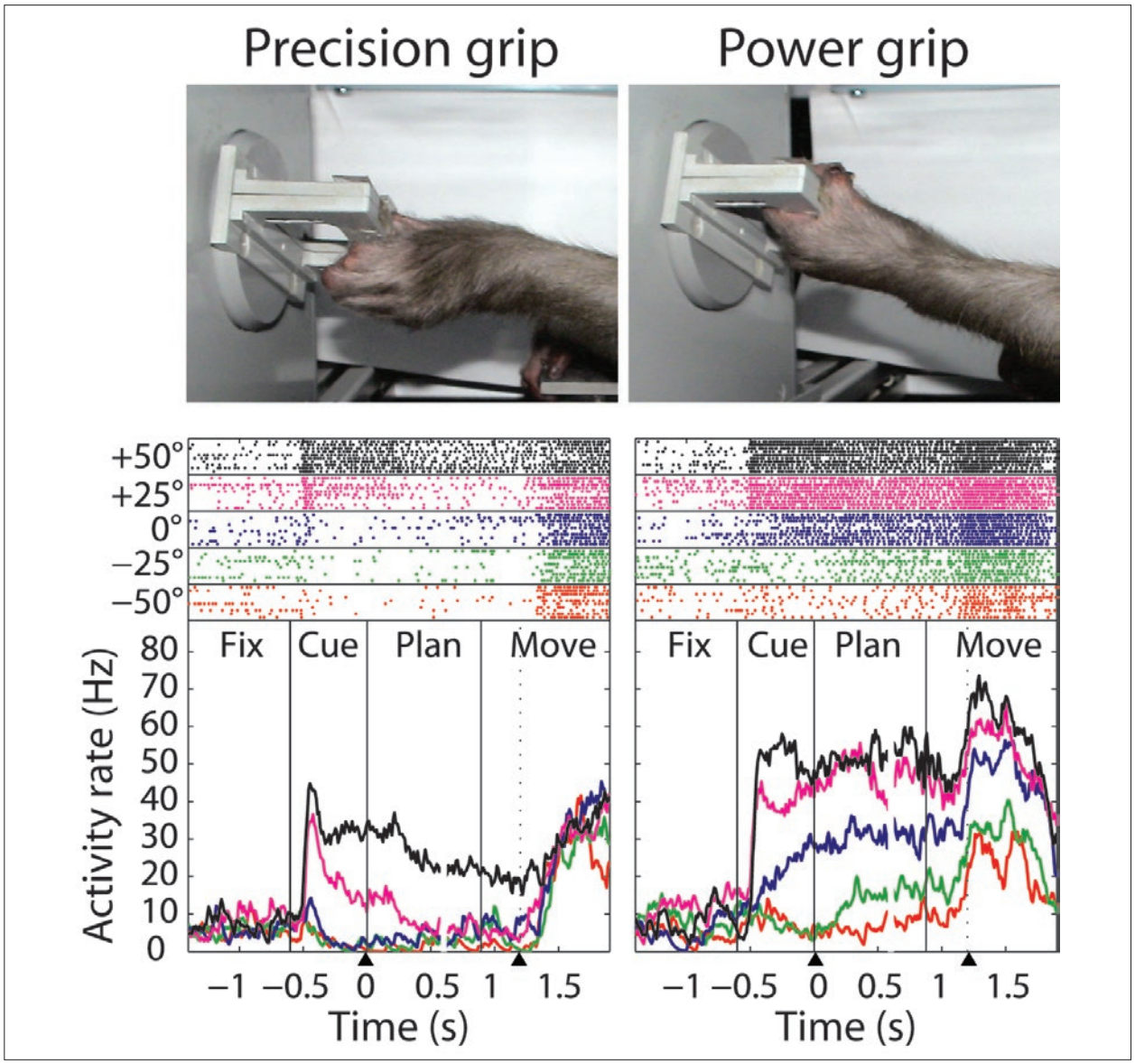


Figure 5: Activity of a single nerve cell of a rhesus monkey during grasping. ***Top panels.*** *The monkey grasps a vertically oriented (0°), or a leftward (-25°, -50°) or rightward (25°, 50°) tilted handle either with a precision grip or a power grip. The task consists of several task epochs: After fixation of a light dot (Fix), the animal sees the handle and the instructed grip type (power or precision grip) is instructed (Cue). After a planning time of ~1 sec (Plan) the animal is requested to execute the movement (Move) and is rewarded for each successful task. Grasp type and orientation are randomly varied in consecutive repetitions.* ***Bottom panels.*** *Activity of an example nerve cell in parietal cortex (area AIP) for both grip types (power, precision) and five object orientations (different colors). Dot rasters illustrate the recorded action potentials, where each row of dots indicates one task repetition. The curves below illustrate the mean firing rate of the nerve cell across all repetitions separately for each hand orientation. The nerve cell shows an increased activity (firing rate) after the instruction period that persists until movement execution. Furthermore, the cell is more strongly active for a power grip than a precision grip, and also more active for a rightward (black and magenta curve) than a leftward oriented handle (red and green curve). Other neurons have different preferences (not shown) so that the neuronal population represents all grip types and orientations (Reproduced with permission from Baumann et al., 2009, Journal of Neuroscience 29: 6436–6448).*

if the signals are stable in time and the brain has enough time to adapt to the decoding errors.

A large unsolved problem of invasive neuroprostheses, however, is that the body reacts against implanted foreign materials and tries to encapsulate them. For most medical implants, like breast implants or cardiac pacemakers, such foreign body reactions are without functional relevance. For recording electrodes in the brain, however, a foreign body reaction means that nerve cells in immediate proximity of the electrode tip are pushed away and their recorded signals get weaker and weaker until they disappear in the electrical noise and no further communication is possible.

Neuroprostheses can also employ signals from the peripheral nervous system (PNS). For this, movement signals are recorded from a peripheral nerve or its innervated muscle. Peripheral neuroprostheses need an intact connectivity from the brain and the spinal cord, which is why this technique is mainly tested in patients with amputation injuries. In these patients, peripheral nerve signals are available at the stump of the limb or at the trunk, from where they can be recorded with invasive or non-invasive methods. Peripheral neuroprostheses therefore use control signals from a peripheral nerve or a muscle and use it to control an artificial effector device.

Such a neuro- or myographcial transmission is robust, but not necessarily fine-graded. Simple signals could be transmitted quite well. However, more complex and coordinated control signals, e.g., as necessary for coordinated hand and finger movements, are much more difficult to implement. For these reasons, peripheral neuroprostheses have been used mainly to control relatively simple prosthetics actuators.

Implantable systems are also of interest for recording peripheral signals, because, once implanted, these implants could work autonomously for a long time without need to be re-fitted every day. This point is quite relevant for practical reasons, in particular for patients who need daily help for fitting these systems.

Scientific foundations

The topic of neuroprosthetics has made tremendous progress in the last 10-15 years. This is related to the immense increase of knowledge about how the brain functions and how neuronal signals can be read out from the brain. To make neuroprostheses possible, groundbreaking progress was necessary in at least three scientific topics: systems neurophysiology, material biocompatibility, and computer science and microelectronics. These fields, as well as medicine, have successfully collaborated in recent years and considerably advanced the field of neuroprosthetics.

Systems neurophysiology: how do we think and act?

Basic scientific advancements about the physiology of the nervous system were paramount for the recent advancements of neuroprosthetics. The discovery of the specific electrical excitability of the brain by Fritsch and Hitzig, 1870, the importance of the nerve cell for brain anatomy by Cajal (Nobel prize in Medicine 1906), brain physiology (Sherrington and Adrian, Nobel prize for Medicine 1932), and the anatomical mapping of brain areas (Brodmann, 1909), all these groundbreaking discoveries caused tremendous innovation in medicine and in particular in neurology and neurosurgery. And many of these fundamental brain functions were attributed, at least coarsely, to individual brain areas.

For neuroprosthetics it was essential to develop a better understanding of how neuronal brain areas process sensory, motor, and internal (e.g., cognitive) information. For example, single neurons in motor and premotor cortex might be characterized by how they are active for a particular grip type (e.g., power grip, like when grasping a branch), whereas they are particularly inactive for another grip (e.g., precision grip, like when grasping a raisin) (Figure 5). These preferred grip types are different for individual neurons, so that within the neuronal population the network can always distinguish arbitrary grip types.

Having understood some of the basic coding patterns of a neuronal population, one can make predictions (i.e., decodings) of intended movements on the basis of an observed single-trial population pattern. For this it is important to know that the variable signals are not isolated, but correlated with each other. For example, motor neurons are also modulated by sensory and cognitive signals representing: attention, intention, reward, etc. This makes precise movement predictions somewhat difficult. However, individual pieces of information can be retrieved from the recorded neuronal population in much more detail than from the EEG. In non-human primates, this technique has been used to develop an artificial gripper that monkeys could use, simply by thought and without using their own hands, to grasp food and transport it to the mouth (Figure 6).

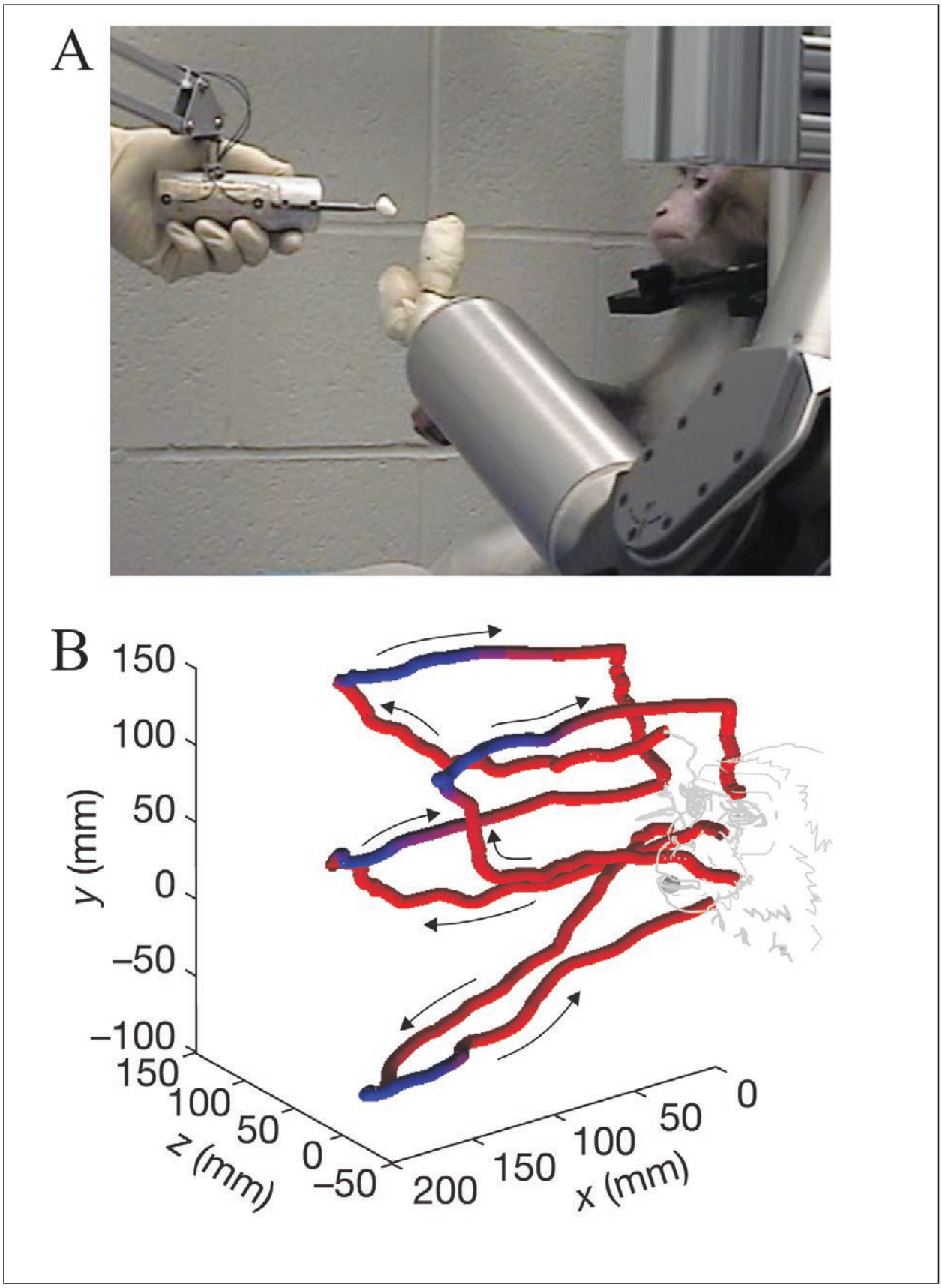


Figure 6: Cortical control of a robot arm for self-feeding using implanted electrodes. ***A:*** *Monkey controls a robotic gripper in real-time that grasps food morsels from various locations and brings it to the animal's mouth.* ***B:*** *Spatial tracking path of the robot hand in four consecutive trials. The color of the trajectory illustrates the state of the gripper (red: open, blue: closed). Note the smooth and goal-oriented movements of the robot (Reproduced with permission from Macmillan Publishers Ltd:Velliste et al:, 2008, Nature 453: 1098–1101).*

Electrodes, biocompatibility, and signal processing

Traditionally, glass- or varnish-insulated metal electrodes have been used in systems physiology to record action potentials of individual neurons. For neuroprosthetics, signals from many different neurons have to be recorded simultaneously. This became possible with the development of electrode arrays that combine individual electrodes to blocks, i.e., to several rows and columns of electrodes. Such electrode arrays can then be permanently implanted in a target area (e.g., motor cortex). Individual electrodes are connected via cable to an external connector block, from which the electrical signals are then amplified and recorded. A flexible cable connection has the advantage that the implanted electrodes are not fixed to the skull and can move along with the floating brain without producing tethering forces. Besides metal electrodes, also electrodes out of silicone or plastic exist, for which methods from microsystems technology have been applied, a technology that is normally employed to build microelectronic chips.

An important point for the development of electrodes is biocompatibility. For neuroprosthetics, this is of eminent importance, since implanted electrodes have a limited lifetime and are encapsulated by the brain as a foreign body. Consequently, a fibrous barrier develops around the electrode that strongly limits or completely destroys the electrode's function. This happens slowly and typically in a timeframe of months and years after implantation. The development of biocompatible materials that evoke no or only minimal foreign body responses is therefore of high relevance for a broader clinical use of implanted neuroprosthetics. Otherwise, patients would need frequent revision surgeries that are associated with increased complication risks (e.g., infections, tissue trauma, etc.).

Furthermore, anti-inflammatory drugs are tested that could reduce the tissue-immanent foreign body reactions. For example, such drugs could be released continuously from a reservoir in the implant. Also, alternative cortical signals are considered for neuroprosthetics applications, which could be recorded even after electrode encapsulation. Such signals could include multi-unit activity, a combination signal of many single neurons, the local field potential, a summation signal of predominantly dendritic activity, and the electrocorticogram (ECoG), which is an EEG signal recorded directly on the cortical surface (Figure 4). All of these signals are currently tested for their robustness, longevity, and capability for decoding.

Computer science and microelectronics

Information technology also made essential contributions to the development of neural prosthetics. In previous years and decades, computing power increased almost exponentially. With this it was possible to build signal processing and data extraction routines with powerful algorithms and simultaneously across many channels, which was impossible before. Furthermore, powerful software packages have emerged (e.g., Matlab, Phyton, Mathematica) that have strongly advanced scientific computing.

Finally, the continuous miniaturization of electronics allows the development of fully implantable systems. This makes it possible to not only record and extract neural data continuously and autonomously, but also transmit the acquired signals by telemetry. Despite high information throughput, such microsystems have only minimal energy consumption, which could also be transmitted wirelessly (e.g., by induction). Together, these features allow the realization of completely implantable systems, which is an important pre-requirement for the long-term use of neuroprosthetics in patients.

The role of animal experimentation

As laid out in the sections above, research with laboratory animals is of central importance for the development of neuroprostheses. Without knowledge gained from animal experiments, and in particular from non-human primates, only very little would be known today about the mechanisms of how the brain generates movements, and the development of clinically usable neuroprostheses would be out of reach. In consideration of the many open questions, it is also clear that research with animals will continue to be necessary in the foreseeable future to extend our knowledge and advance important developments.

Primate research is not only important for the development of neuroprostheses. It is also indispensable for many other research areas, like infection research and degenerative brain diseases. The necessity of such animal research has been documented in many studies. However, it is often injected from ideological sides that animal research, and primate research in particular, is generally useless and unnecessary, and their results cannot be transferred to humans. This is obviously not the case, as can be seen already from the example of neuroprosthetics. Other research topics like the development of deep brain stimulation, understanding severe infection diseases (e.g., HIV/ SIV, hepatitis), genetics, and re-

production medicine similarly depend on research with non-human primates. A ban of primate research would therefore have severe negative consequences for many research areas and for the development of scientific research in the affected countries in general.

Many European countries have a long tradition of regulating animal experimentation (e.g., Switzerland, Germany, United Kingdom, and Holland). These regulations allow necessary experiments with animals while also strongly supporting animal welfare. The administrative procedure for licensing and supervision of animal experiments is complex and demands a detailed presentation of the intended research project as well as an explicit justification. High methodological and ethical hurdles are therefore in place and are strongly enforced at the local, national, and European level.

Furthermore, scientists are inherently interested in optimizing the reliability of their research. In systems neuroscience, for example, this can only be achieved if the animals can participate in the experiment without pain or distress. Careful and slow habituation to the research task is therefore key. Such and similar considerations have led to the continuous refinement of experimental methods (Refine!), the reduction in the number of animals needed to answer a specific scientific question (Reduce!), and the replacement of animal experiments with other methods (Replace!).

This principle of the '3R' (Refine!, Reduce!, Replace!) has been proposed originally by Russell and Burch in 1959. It is currently regarded as the most effective and pragmatic method to effectively minimize suffering in research animals. Researchers have applied this method for many years, even though its public perception has only recently increased (e.g., Basel Declaration, 2010). Such discussions are important in order to inform the public about the importance and the costs and benefits of animal experiments. In this respect, it would be helpful for animal welfare in general, if existing suffering of research animals was discussed objectively, and not ideologically, and in perspective to other forms of animal use in our society (e.g., farm, home, and zoo animals both in terms of quality and quantity.)

Basic and applied research

A large portion of animal research that is now indispensible for the development of neuroprostheses was not conducted for this application in mind, but to investigate basic scientific questions. This is particularly true for the early work. For example, the question how single nerve cells in parietal and premotor cortex contribute to the planning and execution of limb movements is of basic scientific interest, and originally this question was not posed with a specific application in mind. Instead, these results were a consequence of basic scientific interest, which produced these important impulses for practical applications.

It should be noted that it is a general property of the scientific method that it is impossible to predict future scientific knowledge or discoveries. It is also impossible to pursue them in a goal-oriented fashion. Basic research has potential for applications and vice versa, applied research can lead to unexpected basic scientific results. A strict classification of research in basic or applied science is therefore neither possible nor useful.

Consequently, the value of an animal experiment cannot be determined immediately. It can take quite long until the true value of an experiment might become apparent. Furthermore, negative results can also increase knowledge. Experimental results are like stones of a mosaic: each stone is only part of a larger picture. Therefore, the most crucial factor for evaluating a scientific experiment is not the obtained result. Instead, it is the importance and originality of the scientific question, the quality of the experimental design, and the potential, but not the actually obtained increase of knowledge.

Conclusion

Neuroprostheses have recently made much progress in both non-invasive and invasive methods. This progress has been possible because of major developments in neurophysiology, electrode technology, and computer science. They render the idea of an efficient and applicable brain-machine interface for paralyzed patients more and more realistic. Animal experiments, in particular with non-human primates, have contributed crucially to this progress. Because of these continuous developments, it can be assumed, with guarded optimism, that more and more patients can benefit from these rapid developments in the future.