Laboratory Animal of the Year:

The Mouse in Parkinson's Research

Photo: Filo, iStockphoto

Every years since 2003, the organisation People for Animal Rights Germany has presented the "Laboratory Animal of the Year". The goal is to publicise animal experiments carried out on a specific species. We also show what opportunities already exist for research without the use of animals. 2019 is dedicated to the mouse in Parkinson's research; this species is much deserving of the attention, because about two thirds of all animal experiments are carried out on mice.

Menschen für Tierrechte
Bundesverband der Tierversuchsgegner e. V.

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Excursus: Parkinson's Disease

Parkinson's disease (earlier also known as shaking palsy) is considered the second most common neurodegenerative disease after Alzheimer's disease. It typically begins between the ages of 40 and 70 years, less often earlier[1,2]. In Germany it affects between 250,000 and 280,000 people. Worldwide, 4.1 million people are reported to suffer from Parkinson's disease, with numbers rising[3,1]. The disease affects men more often than women. In about 90% of cases, the cause is not known or not sufficiently known, with only 10% being due to genetic reasons[2,4]. Earlier encephalitis, environmental toxins, pesticides or medicines may also play a role.

In Parkinson's disease, the death of dopamine-producing neurones in the substantia nigra (in the midbrain) cause typical motor symptoms such as tremor at rest, slowness of movement, muscle rigidity or rigid facial expressions[5,6,7]. In addition to the death of the nerve cells, so-called Lewy bodies accumulate in the nerve cells of the substantia nigra[2]. Damage to Complex I in the electron transport chain of the mitochondria (the power plants of the cells) seems to be central to the development of the disease, but scientists do not know exactly which individual steps lead to neurodegeneration and subsequently to Parkinson's symptoms[4]. The mutations implicated in developing Parkinson's disease are mainly to be found in the five genes SNCA, PARK2, PINK1, DJ-1, LRRK2 or UCHL-1. These lead to reduced mitochondrial activity and oxidative stress, which can cause inflammation and death of the nerve cells[8]. The protein α-synuclein, which normally regulates dopamine release in neurones, accumulates as Lewy bodies in the nerve cells[9].

Parkinson's disease is usually only diagnosed after 60% of the dopamine-producing neurones have perished[10]. Currently available treatment methods cannot halt the disease and there is no cure yet. One can only treat the symptoms to make the disease more bearable for the patient[11]. The drugs currently available are used to try and counteract the dopamine deficiency in the brain or to restore equilibrium between the neurotransmitters. However, this does not always succeed, or the effect diminishes with time[12]. Levodopa (L-dopa), a drug discovered as early as the 1970s, other drugs such as safinamide[13] and deep brain stimulation cannot prevent the disease's progression.

Parkinson's disease is 1.5 times more common in men than in women. Scientists and physicians distinguish between Parkinson's disease itself and Parkinsonism. In Parkinson's disease, dopamine production is disturbed, whereas Parkinsonism is a clinical syndrome with symptoms characteristic to, but not caused by, Parkinson's disease itself and has a range of causes including neurodegeneration or medication.

Photo: stevepb, Pixabay

Patients with advanced Parkinson's disease who are treated with drugs such as L-DOPA develop side effects such as dyskinesia (abnormal movements) and psychosis[11]. Physicians therefore administer other dopamine agonists that directly affect the nerve cell receptors. However, these are also associated with neuropsychiatric problems, such as hallucinations, severe insomnia and addictive behaviour. They are also not as effective against the Parkinson's symptoms. Further drugs such as apomorphine are under development[14]. A more recent approach

Levodopa (L-DOPA) is still the gold standard for treating the symptoms of Parkinson's disease.

Photo: dertrick, Pixabay

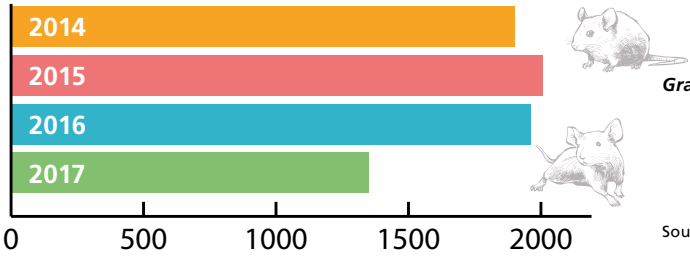
is the antidiabetic agent exenatide, which can stabilise dopamine-responsive nerve cells in the brains of at least younger patients[12]. Deep brain stimulation requires less medication. An electrode is implanted directly into the brain and generates high-frequency pulses via an internal pulse generator. However, its applicability is limited to certain groups of patients, it is only partly effective and personality changes occur. Transplanting new dopamine-responsive nerve cells is a method hoped to be viable in the future[15], however the method involves ethical concerns, a lack of donor cells and poor survival of the transplanted cells in the recipient tissue. The procedure is also not standardised[16].

Mice in Animal Experiments

The mouse has been laboratory animal no. 1 in Germany for decades. In 2017, 1,350,727 mice were used in animal studies. Of these, 53% were genetically modified, 11.5% with a debilitating phenotype[17].

Mice are most often used for research into Parkinson's disease. Photo: Filo, iStockphoto.

60% of laboratory mice were used in basic research and 18% in applied and translational research. The latter aims to expedite implementation of a procedure or therapy. About 15% of the mice suffered in tests required by law. The remaining mice were used in education, vocational training and ongoing education, in the context of conservation issues or for maintaining breeding colonies.



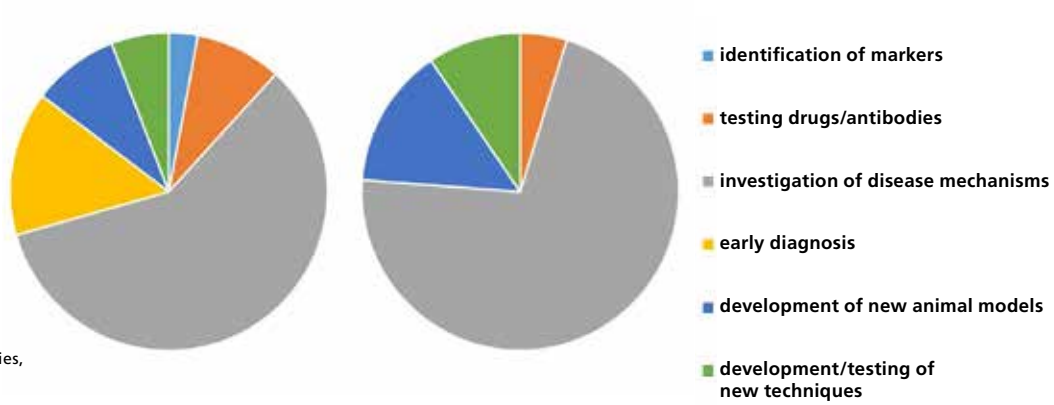
Graph 1: ***Mice used for research purposes in Germany between 2014 and 2017. The animals were used in accordance with Section 7, Subsection 2 of the German Animal Welfare Act, including animals killed for the removal of organs for scientific purposes in accordance with Section 4, Subsection 3 of the German Animal Welfare Act.***

Source: Laboratory animal statistics from the German Federal Ministry of Food and Agriculture.

The official laboratory animal statistics do not allow any direct conclusions regarding the number of mice used for research into Parkinson's disease, with the actual number hidden somewhere in the categories "human nervous system research" and "mental diseases", which include Alzheimer's and dementia. For this reason we took a closer look at the non-technical summaries (NTS) that must be published within 12 months in a database at the German Federal Institute for Risk Assessment (BfR). An NTS documents approved animal testing, not the actual number of animals used[18]. The number of animals approved is generally valid for 3-5 years. The descriptions are not always clear and often sketchy.

Mice in Parkinson's Research

The mouse is THE experimental animal in basic and applied research into Parkinson's disease. To a lesser extent, rats (6,808 in 2017, 5,216 in 2018), zebrafish (3,968 in 2017) and sheep (20 in 2017) are also used, as well as some cynomolgus monkeys for statutory drug testing for the treatment of Parkinson's disease (96 in 2017, 17 in 2018, sources: NTSs in www.Animaltestinfo.de, BfR)[18]. The experiments with mice are conducted mainly to investigate disease mechanisms, followed by tests for the development of early diagnoses. The mice used were either genetically engineered or treated with a neurotoxin such as MPTP in order to trigger mutation.



Graph 2:
Main objectives of animal experiments with mice in 2017 (left) and 2018 (right) in Germany.

Source:
Non-technical project summaries, AnimalTestInfo, BfR.

In 2017 and 2018, just under two thirds of all "Parkinson's" mice were used for investigating disease mechanisms (grey). The second most frequent goal in 2017was early diagnosis (yellow). In 2018, additional animal models were developed and new techniques were tested. By contrast, early diagnoses and markers played no part.

Mice in Everyday Laboratory Life

House mouse (Mus musculus) out and about.
Photo: Georg Wietschorke, Pixabay.

In nature, mice live in stable social groups and need constant interaction with other members of their species. They communicate via scents and sounds, especially in the ultrasound range[19]. Tests have shown that male mice encountering females sing veritable songs in the ultrasound range between 30 and 110 kilohertz, with different syllables and repetitive sequences[20]. By comparison, the upper threshold of human hearing is around 20 kilohertz.

In the laboratory, on the other hand, a mouse's life looks completely different. Mice can be bred quickly and are kept in large numbers in small spaces. According to Annex III, Section B of Directive 2010/63/EU on the protection of animals used for scientific purposes, mice held in stock and during laboratory experiments must be provided with an area of 60 to 100 square centimetres per animal with a height of 12 centimetres. The Directive allows a breeding pair an area of 330 square centimetres with a height of 12 centimetres[21]. These conditions are even close to suitable for the animals, because the mice cannot fulfil their needs, such as sufficient exercise, climbing and gnawing. in recent years, scientists have increasingly addressed the subject of improved housing conditions, and the small plastic cages now mostly include litter, houses, occupation material for the animals.

Mouse "Models"

In biomedical research, the term "animal model" is used for animal species or breeding lines that can develop symptoms of a particular human disease. These animals are then examined in place of humans with regard to symptoms and processes of the diseases. However, results from experiments with such animal models are not readily transferable to humans.

Hundreds of mice are bred for experiments. Here, many animals are kept in plastic tubs in mobile stands during the animal experiment.

Photo: C. Hohensee.

In Parkinson's research, scientists distinguish between etiological and symptomatic animal models. Etiological animal models bear exactly the mutations that are also known in humans. Researchers want to use this to understand the development of the disease, and find biochemical and cellular changes. Symptomatic models only represent the external appearance of the human movement disorder. They are used to test drugs and discover anatomical and physiological processes[22].

Most scientists agree that none of the available generated models can reproduce all aspects of the disease. The animal models currently available are also not sufficiently reliable or informative[22]. Nevertheless, they are repeatedly produced and used.

Genetically Engineered Mice

Numerous genetic engineering methods routinely use mice to produce disease "models". A globally established method called CRISPR/Cas9 plays a special part. CRISPR/Cas9 is a gene editor that opens the DNA strand at the desired site and makes it possible to cut out, insert or even alter only one nucleotide.

Transgenic Mice

Mice with a previously non-existent gene or gene sequence inserted into the genome are called "transgenic". The foreign genetic material can come from mice, other species or even from humans. In the case of Parkinson's disease, a human gene sequence or a mutated gene is usually targeted to a site of the genome ("knock-in"). The aim is to create human diseases in the mice in order to gain insights or to test remedies.

Knock-out Mice

Another method for altering the genome of the mice is the so-called knock-out technique. This involves permanently deactivating or removing specific mouse genes ("knock-out mice")[23]. In some cases, the gene knock-out is not permanent, but "only" causes an epigenetic change via an additional protein that prevents reading of the gene sequence. One variant is gene silencing: Small interfering RNA pieces (siRNA) or short hairpin-shaped pieces of RNA (shRNA) do not deactivate the gene itself but rather a gene's messenger RNA that serves as a template for translation to a protein[23].

Large Consortia for Engineered Mice

The International Mouse Phenotyping Consortium (IMPC) is a worldwide scientific project[24]. Its goal is the production and phenotyping of targeted knock-out mutant mice for more than 20,000 known and suspected mouse genes in embryonic stem cells. The IMPC is an association currently composed of about 20 international research institutions and five funding members from 11 countries, including from the European Union. The research facilities include universities, Charles River Laboratories, Inc., and The Jackson Laboratory. Germany is represented by the Helmholtz Zentrum München (German Research Center for Environmental Health). A database with 53 records of knock-out mice is available for use in Parkinson's disease research. The database includes information on the modified genes PARK1-4, PARK7, PINK1, but also altered genes that encode other proteins and thereby trigger neurologically induced movement disorders, as well as data on early stages of Parkinson's disease and Parkinson-like diseases. Members can access information free of charge for their further research, as engineered embryonic stem cells, vectors, data, protocols and analysis tools.

There is also a European programme for the rapid production of mutant mice, the European Conditional Mouse Mutagenesis Program (EUCOMM). EUCOMM maintains and updates a database on genetically engineered embryonic stem cells[25]. From Germany, the Institute for Experimental Genetics, the Institute for Developmental Genetics of the Helmholtz Zentrum München and Goethe University Frankfurt are involved. Researchers at the Helmholtz Zentrum München are developing knock-out mutants in which the mutation in the mice only triggers Parkinson's under controlled conditions. The Helmholtz Zentrum München also breeds mice in which the common Parkinson genes such as LRRK2, PINK1 or DJ-1 are introduced into the animals[10,26].

Commercial Mouse Production

The company GenOway based in Lyon specialises in the production of genetically engineered mice, rats and cell models[27]. The company collaborates with universities and the pharmaceutical companies such as Merck and Charles River in the development and commercialisation of rodent models. GenOway has developed a mouse model for Parkinson's disease, in which the Pla2g6 gene thought to be responsible for certain Parkinson's symptoms has been manipulated[28]. Mutations in the gene alter the phospholipid metabolism in the nerve cells[29].

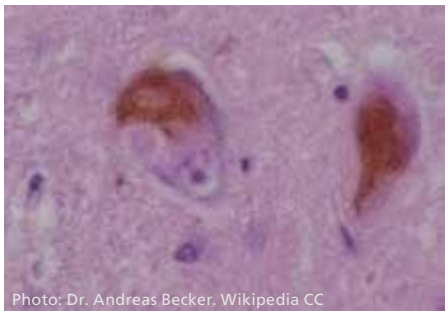
Charles River, on the other hand, support scientists with their own development, characterisation, maintenance and dissemination of a Parkinson's mouse model. Their "ready-to-use" mice do not include a model for Parkinson's disease. Charles River also market their JAX™ mouse in Europe in collaboration with The Jackson Laboratory in Bar Harbor, Maine. The Jackson Laboratory offers 202 different mouse strains developed for Parkinson's research[30,31]. Some are available as live mice, bred and kept in colonies. There are freeze-dried embryos of rare strains or mouse sperm can be ordered[32]. Scientists also use the implantation of cryopreserved embryos (stored in liquid nitrogen) for in vitro fertilisation[33].

Genetically Induced Parkinson's Symptoms

Less than 10 percent of Parkinson's cases are genetic. Nevertheless, animals are used in research into the disease. Five genes are central: SNCA, PARK2, PINK1, DJ-1 and LRRK2. Some of the genes are autosomal dominant, so a mutation on one of the paired genes is enough to trigger the disease. Such a mutation does not occur on a sex chromosome and is therefore independent of the biological sex. The autosomal dominant genes include SNCA (for the protein α-synuclein) and the PARK8 gene for an enzyme called LRRK2 (leucine-rich repeat kinase 2).

SNCA

Many studies deal with the protein α-synuclein, which accumulates as so-called Lewy bodies. These are thought to be a cause of Parkinson's disease, as they are responsible for the death of the nerve cells[9]. A point mutation on the gene is sufficient to trigger the process. The point mutations induced in the gene SNCA in laboratory animals are called A53T, A30P or E46K. The result is a higher level of α-synuclein, which forms fibrils and thus boosts the formation of Lewy bodies[33].



Lewy bodies in melanin-containing nerve cells of the substantia nigra in a Parkinson's patient.

Several α-synuclein transgenic Parkinson's mouse lines have been created, but none of the models has accurately represented Parkinson's disease. The scientists were able to trigger dysfunctions, but there was no progressive loss of dopamine-responsive nerve cells. Therefore, scientists have also introduced genetic material from degenerated proteins (prions) into the transgenic mice to trigger the full extent of α-synuclein pathology as seen in humans[35].

PARK8

PARK8 is the genetic template for a large enzyme with many binding domains, called LRRK2. The enzyme localises to cell membrane structures. The mutation inactivates the enzyme function[36,37]. This hinders the cellular transport process and influences recycling within the cells as well as neurotransmitter transport. Although LRRK2 mice have abnormalities in the nigrostriatal system and behavioural abnormalities, they produce a very mild phenotype with minimal neurodegeneration. The models are not considered robust[35].

In autosomal recessive genes, both chromosomes must be affected by the mutation to trigger Parkinson's disease. The genes best characterised are Parkin, DJ-1 and PINK1. For each of these genes, at least one knock-out mouse model has been developed, but none of the breeds displays the characteristic pathology in the substantia nigra, which is why the knock-out mice are used to study early neurodegenerative changes[35].

PARK2

The gene PARK2 encodes an enzyme called E3 ubiquitin ligase, also known as "parkin protein". In its normal state, parkin labels defective proteins in dopamine-forming neurones by attaching a signal molecule (ubiquitin) to the protein, so that it can be removed. This does not take place when the gene is mutated. Defective proteins accumulate in the cells, causing cell death[38,39].

DJ-1/PARK7
The gene DJ-1 (also known as PARK7) encodes another enzyme. This allows the production of D-lactate and glycolate, which are vital for the effectiveness of the mitochondria and therefore of the nerve cells[40,41].

PARK6/PINK1
The gene PARK6 is the template for the enzyme PINK1, which interacts with parkin. PINK1 binds to defective mitochondria, accumulates on the outer membrane of the mitochondrion and activates the enzyme function of parkin (see above). Parkin then attaches a signalling molecule to induce breakdown of the mitochondrion[42].

Researchers suspect a correlation between genetic variants and the influence of chemicals in the environment[34]. In order to investigate this, the genes described above were engineered in mice, but the mice were often additionally exposed to neurotoxins, compounding the suffering.

Chemically Induced Parkinsonism

Rotenon
The insecticide rotenone, which is banned in the EU Member States[43], causes Parkinsonism in mice, rats and other animals. It interferes with the electron transport chain in mitochondria[44]. The substance is often systemically administered to laboratory animals via an osmotic micropump[34]. This causes degeneration of dopamine-producing nerve cells and accelerates deposits in the nerve cells of the substantia nigra, similar to Lewy bodies in Parkinson's disease. It also leads to impaired digestion, impaired sense of smell and movement disorders, many symptoms that also occur in Parkinson's patients.

Derris elliptica from Borneo, also known as tuba root. The plant contains the substance rotenone, which is not approved as an insecticide in the EU Member States, but is allowed in other countries.

Photo: Wibowo Djatmiko, Wikipedia CC BY-SA 3.0

MPTP
Originally a by-product of a synthetic opioid, discovered when it caused Parkinsonism symptoms in drug addicts[43]. MPTP is first converted in the brain and then preferentially absorbed into dopamine-responsive nerve cells. Once in the nerve cell, it blocks the mitochondrial electron transport chain and leads to the formation of toxic oxygen molecules known as reactive oxygen species (ROS). Mice treated with MPTP display cell loss in the substantia nigra and motor impairment[35].

6-OHDA

The neurotoxin 6-hydroxydopamine (6-OHDA) is one of the world's most widely used neurotoxins for causing artificial nerve damage to animals for research into Parkinson's disease[45]. It is taken up by the dendrites and synaptic buttons of noradrenaline-producing and dopamine-producing neurones, resulting in damage to the nerve cells and their death[45]. 6-OHDA cannot pass the blood-brain barrier, therefore the neurotoxin is administered by "intracerebral infusion" after drilling the skull open (stereotactic brain surgery)[48,49]. 6-OHDA preferentially targets the dopamine-responsive neurones in the substantia nigra, where it is toxic and ultimately kills nerve cells[35]. In the test, however, it does not develop the same pathology in the cells as is observed in Parkinson's disease. The toxin does not seem to work through the same molecular mechanism[35]. Researchers nonetheless intend to use animals treated this way to investigate the mechanisms of neurodegeneration, the behavioural deficits and motor damage.

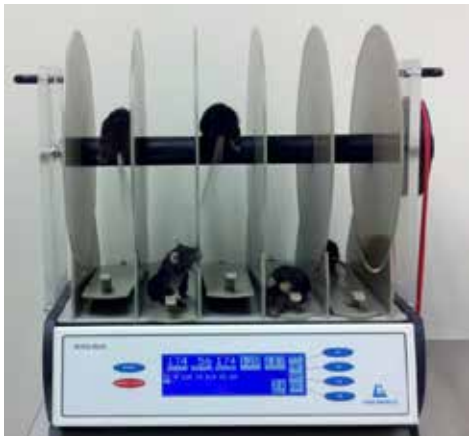
Newer Modifications

There are various other genetic modifications, such as the FBX07 knock-out[49,50,51] or mice with a modified immune system (so-called Siglec 11 transgenic mice)[52]. It is known that inflammation plays a part in Parkinson's disease[53].

Engineering is Followed by Tests

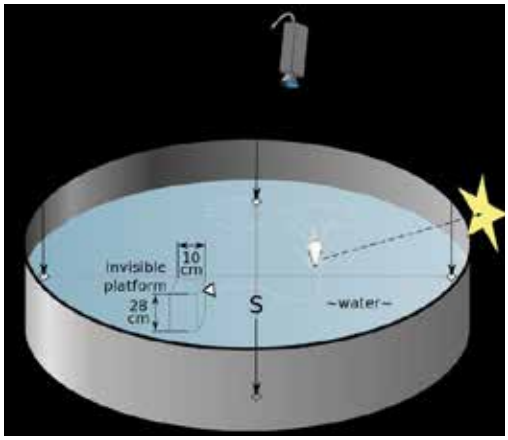
After creating an animal "model", motor and behavioural tests are conducted to investigate how successful the modification is, the drug is then administered if necessary and an improvement of the symptoms is also likewise investigated in motor and behavioural tests.

In the Rotarod performance test, the mice are placed on a rotating wheel and observed for 5 minutes. The wheel rotates at 2 to 7 revolutions per minute and can also be operated at variable speeds. The aim is to assess sensorimotor coordination and resistance to fatigue[54].



left: Mice on/under the rotarod wheel. Three animals are already showing signs of fatigue and have fallen off the rotating axle.

Photo: Bmouzon, Creative Commons BY-SA 4.0



right: Morris water navigation test. The mice must find a platform 1.5 centimetres under the surface of murky water in a large circular pool and be able to orient themselves. In the case of Parkinson's disease, this is not possible because the disease triggers hippocampal atrophy, impairing spatial learning (6).

Illustration: Hunter.ar, Creative Commons BY-SA 4.0

After MPTP treatment, an aversion stimulus may be additionally administered in the form of a mild electric shock when the mice fall off the wheel[55]. The time the animals remain on the wheel is analysed.

In the Morris water navigation test, the mice must find a platform located 1.5 centimetres below the surface of murky water in a round swimming pool and be able to orient themselves. In the case of Parkinson's disease, this is not possible because the disease triggers hippocampal atrophy, impairing spatial learning[6].

The open field test investigates spontaneous activity, exploratory behaviour, and anxiety in mice induced by Parkinson's disease. The activity is recorded with a video camera over a period of time, and the distance measured that the mice cover during half an hour in the open field[55].

Mice in Parkinson's research may be made to walk on a grid to assess gait after an induced unilateral 6-OHDA lesion. The genetically engineered mouse is placed on a metal grid with a mesh spacing of 2.5 cm. Over a period of five minutes, the number of times a mouse attempts to place weight on a foot falling between the wire grids is counted[56].

In the hanging-grip test, mice are placed on a metal grid with 2.5 centimetre mesh spacing for a period of one minute. The grid is then turned upside down to observe how long the animals can hang onto the mesh before they fall into the cage below[57].

There is also the pole test, where mice are placed on the top of a 40 centimetre vertical pole to observe how long they can stay on the pole and how they descend.

Scientific Criticism of Models

Scientists agree that no single model is able to simulate human Parkinson's disease[22,58,59]. To date, scientists have found that Parkinson's is the result of a complex interplay of genetic causes and environmental factors[59]. Despite the plethora of animal models available, a complete understanding of Parkinson's has not been achieved. Nevertheless, many researchers currently regard animal models as a necessity. They counter criticism of the meaningfulness of individual models by advocating a combination of several individual models[58].

At the conference "Tierversuche: Geht's auch ohne?" ("Animal testing: Can they be done away with?") hosted by the Volkswagen Foundation in February 2019, Prof. Bernhard Hiebl, Professor for Laboratory Animal Science at the University of Veterinary Medicine Hannover, criticised the validity and transferability of animal experiments to humans. There were more than 50 unsuccessful human clinical trials of Parkinson's drugs, all of which had previously been successful in animal models[60]. Nearly 60 years after its discovery, levodopa is still the "gold standard" for treating patients with Parkinson's disease[59].

Insufficient reproducibility of research results is a major problem, especially when phenotypes only manifest subtly or there is high individual variability[44]. There are critical researchers who believe that the approach using engineered animals is wrong, because researchers usually target their animal models to degeneration of the substantia nigra. There is criticism of the fact that this does not address the cause of processes in Parkinson's disease but rather the result. Animal models can reproduce neither the healthy nor the diseased human brain[61]. Additionally, in about 90 percent of patients, Parkinson's disease is not the result of genetic mutation or polymorphisms. Despite this, researchers around the world continue to develop countless genetically engineered animal models solely for the purpose of reproducing Parkinson's phenomena.

Most drug tests for treating Parkinson's disease in humans are conducted on young male mice and rats. This is problematic because Parkinson's disease is usually an age-related disease[44]. Researchers also criticise that nerve agents such as 6-OHDA neither trigger the same pathology in the cells nor work through the same mechanisms as observed in human Parkinson's disease[36]. One background for the preferential use of neurotoxic models such as MPTP and 6-OHDA in preclinical studies may be the relatively low cost and rapidity of degeneration processes in animals[44]. However, symptoms induced by neurotoxins in animals are not consistent with Parkinson's disease[48]. The neurodegeneration is generated very rapidly within a few days and the animals do not develop the Lewy body deposits that are characteristic to Parkinson's and consist of α-synuclein. The main mechanism that is triggered in the animals is the blockade of mitochondrial Complex I. Often the symptoms are only induced on one side of the body in order to detect motor impairments at all[49]. In addition, often only a single point in time is used to determine a study result. Therapeutic test substances are even administered before or during development of the toxin-induced damage[44]. This has little to do with reproducing a human neurodegenerative disease.

Reproducing only some of the complex disease features of human Parkinson's disease is a major challenge. Whilst non-motor symptoms such as sleep disorders, depression, hallucination, anxiety and psychosis are becoming increasingly important as early diagnostic markers for Parkinson's disease, they are poorly characterised in rodents[44].

There is also criticism of the motor tests: The Morris water navigation test, one of the most widely used tests for measuring hippocampus-dependent learning, cannot be used in animal models for Parkinson's disease, because possible positive test results may not be due to impaired memory but rather to bradykinesia – slowness of movement typical to Parkinson's disease.

Are There Alternatives?

Cell cultures offer at least some advantages compared to directly studying Parkinson's disease in patients or with animal models: Multiple modifications can be tested simultaneously using cells, the experiments have a short duration and there are few ethical and regulatory concerns. Genetic and pharmacological interventions are comparatively simple, and imaging and biochemical analysis are easy to handle[62].

Here are some examples of applied non-animal methods in Parkinson's research:

1. Endogenous Neural Stem Cells for Translational Research

Parkinson's disease is known to damage the mitochondria in nerve cells. A team of scientists from the Hertie Institute for Clinical Brain Research and the University of Tübingen therefore investigated whether mitochondrial dysfunction is just an accompanying phenomenon or actually triggers Parkinson's disease. They took skin cells from Parkinson's patients, dedifferentiated them to stem cells and used them to produce nerve cells. The cells showed damage in the so-called GBA gene, the most common risk gene for Parkinson's disease. The gene is the template for an enzyme involved in lipid metabolism. Damage leads to limited mitochondrial function and thus limited energy production. The scientists administered nicotinamide riboside (a form of vitamin B3) to the cell culture. Nicotinamide riboside is a precursor to the intracellular cofactor nicotinamide adenine dinucleotide (NAD), which stimulates cells to form new

mitochondria. The cell culture formed new mitochondria and energy production increased. The researchers concluded that nicotinamide riboside may be useful in the treatment of Parkinson's disease[63].

2. Genetically Modified Cell Cultures in Basic Research

Researchers from Johns Hopkins University in Baltimore, Maryland, discovered a genetic mutation that connects the onset of Parkinson's disease and plaque development in the brain. They modified human nerve cells using the gene editing technology CRISPR/Cas9 to mutate the gene GBA1, and observed that this led to the accumulation of a specific fat molecule. At the same time, the number of α-synuclein tetramers fell.

Fat molecules in the cell membrane bind proteins in a certain way. When GBA1 mutates, the composition of the fat molecules in the cell membrane changes and there is an accumulation of fat in the cells. The researchers suspected that the levels of fat cause protein fragments (α-synuclein) to aggregate in the brain and form Lewy bodies. This impairs learning, behaviour and mobility. Healthy cells also contain α-synuclein, but normally the individual proteins combine to form groups of four, so-called tetramers. This makes them more resistant to aggregation in the brain. In Parkinson's disease, however, single α-synuclein binds to cell membranes, making it impossible for neurones to communicate properly. The scientists were able to stop this process by adding a drug used for treating two rare hereditary metabolic diseases[64].

3. In Vitro Disease Models

Despite the development of many models, researchers have not yet been able to get to the bottom of Parkinson's disease, the use of in vitro disease models to search for new treatment options is now commonplace around the world. The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) also supports the development of in vitro disease models using patients' own cells. In the DACaION project, for example, scientists from the University of Hamm-Lippstadt will collaborate with the Max Delbrück Center for Molecular Medicine in Berlin, where the production of dopamine-producing nerve cells responsible for the disease will be examined in vitro. The insights gained are to be used to optimise and standardise cell cultures. Dopamine-responsive nerve cells from Parkinson's disease patients in a previous clinical study will then be used to develop disease models, with which small molecules can be tested for their therapeutic efficacy. The DFG will provide the project with €276,000 in funding over a period of three years[65].

4. Microfluidic Systems for Parkinson's Research

A scientist from the Institute of Physical Biology at Heinrich Heine University Düsseldorf is currently researching the transformation of the protein α-synuclein into a α-sheet stage, which also plays a role in Parkinson's disease. For this purpose, he develops and uses novel biosensor-based methods and so-called microfluidic systems to observe the behaviour of minute amounts of dissolved protein on microchips under various conditions[66].

A Dutch-Luxembourgish collaboration has just started developing a "midbrain-on-a-chip", with which the scientists intend to further explore the causes for Parkinson's disease. The tissue in organoid form is derived from induced pluripotent stem cells obtained from Parkinson's patients. A particular challenge lies in the oxygen and nutrient supply of the spherical organoids; the scientists have developed a chip system, with which they can control the nutrient flow in long-term cultures of the miniaturised midbrains[67].

5. Other Cell Models in Basic Research

Other scientists have also been researching the biochemical modifications of α-synuclein for some time. In Parkinson's disease, the nerve cells contain more α-synuclein than healthy cells do. An excess of this protein causes the deposits in the cells typical to the disease. Phosphorylation of the protein (addition of a phosphate group) is known to have a protective effect, and nitration (attachment of a nitrogen oxide) may have a damaging effect. Basic researchers at the Cluster of Excellence CNMPB Göttingen use yeast cells to study these cellular mechanisms and have analysed a complex mechanism that is essential for cell mortality in Parkinson's disease[68].

6. In Silico

Parkinson's disease can also be researched in silico using computer simulations, mathematical algorithms and machine learning. However, this requires reliable human-specific data, not results from animals. Computer programs could be used to predict the interactions between molecules and cells via signal transduction pathways through to the outcome in entire biological systems[61].

In Vitro Methods for Innovating Modern Pharmaceuticals

In vitro models definitely provide important innovative screening opportunities to identify new potential therapeutics. This could also be conducted in high-throughput, which is not possible using animal models. Cell cultures can help to find the desired genotype, proteins or micro-RNA molecules, which can regulate the translation of the DNA information to a protein. In cultures of SNCA mutant neurones, for instance, researchers discovered that a particular signalling pathway was inhibited. Using high-throughput screening, they were able to identify molecules that could nonetheless activate the inhibited gene reading and translation to a protein, and thus repair the defect[69]. There are many such examples.

How Should In Vitro Methods Be Classified?

Parkinson's disease is human-specific, very complex and multifactorial. In this context, new cell-based "human models" are invaluable resources, as they relate to the human organism. Researchers can approach the disease in a patient-specific environment. Like an animal model, however, models using patients' own stem cells have their limitations. There is only a small section of the human organism available, and that cannot simply be used to deduce a coherent physiological organism. Cultured cells are reduced systems that allow us to quickly answer specific questions and elucidate signalling pathways or mechanistic details. Reduced systems, like a Parkinson's model, cannot represent all aspects of Parkinson's disease[62]. Scientists therefore support the idea of piecing together the disease mechanism with a multitude of different individual systems[70]. To this end, new human-specific approaches are favoured, based on a combination of induced pluripotent stem cells, 3D patient cell cultures, in silico

analyses, non-invasive human brain imaging and modern AOP (Adverse Outcome Pathway) approaches. Together these could accelerate the discovery of suitable therapies[61]. An AOP is defined as a series of events that start with a molecular initiating event (MIE), such as the inhibition of an enzyme in the cell; it progresses through a series of key events, such as the alteration of a signal transduction pathway, ultimately to a negative result, such as the death of nerve cells[71].

The high failure rate in the development of pharmaceutical drugs proves that the current approach using genetically engineered animals and animals impaired by administered substances – in this case mice – is the wrong approach.

Outlook

The developers of pharmaceutical drugs are very interested in human-specific procedures. Scientists are also working on solutions for the especially complex systemic approach. Successes have been made in the fields of stem cell research, chip technology and imaging techniques. Any new technique contributes to speeding up developments as a whole; once the procedures have been developed, they can help to reduce animal testing beyond the area of risk assessment. For this to happen, they must be made widely known on the one hand, financially feasible on the other. New technical possibilities can pave the way for completely new research approaches in general, both in basic and applied research. Adequate funding is and remains decisive for the rapid development of efficient animal-free methods, but reality still tells a different story: The bulk of research funding is still spent on research using animals, which is why People for Animal Rights Germany is calling for an immediate reallocation of funding, so as to approach the goal of abolishing animal testing defined in Directive 2010/63/EU as purposefully and speedily as possible.

Implementing a Package of Measures

People for Animal Rights Germany campaigns on scientific, political and social levels for the abolition of animal experiments. The laboratory animal of the year is one means by which the organisation informs the public and presents concrete solutions. In order to achieve this goal, People for Animal Rights Germany has compiled a comprehensive catalogue of measures, and demands that politicians develop an overall strategy for animal-free science. At the top of the list of necessary measures is a substantial expansion of animal-free research, in particular by increasing research funding in Germany and the EU. Anyone seriously interested in successfully developing new methods must have a budget for this branch of life sciences that at least equals funding for research using tests on animals. New criteria for the allocation of funds and the promotion of young scientists are equally indispensable. This is why chairs and professorships for animal-free science, teaching and training must be established. Another important measure is a ban on specific animal experiments. EU law already allows the prohibition of certain animal experiments, even if no animal-free methods are available. This includes in particular the unconditional prohibition of animal experiments that cause severe stress. A drastic reduction of the time authorities take to test and approve animal-free methods must be facilitated. This phase currently lasts between six and fifteen years! As part of its project InVitro+Jobs, People for Animal Rights Germany presents animal-free methods and scientists, contributing to the promotion of important information and networking in this field.

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