Biomedical Primate Research Centre
Annual Scientific Report 2022

Welcome

Join our journey through health research and alternatives

You're looking at a 3D presentation of beta-amyloid protein that disrupts nerve cells in a brain with Alzheimer's disease

(Source: Shutterstock)

Biomedical Primate Research Centre

Contents

Monkey Research

- Explained -

Welcome to the 2022 annual scientific report from Biomedical Primate Research Centre (BPRC). In this report our scientists inform you about their work with monkeys and their most important scientific findings. As you will see, our work covers many different aspects, collaborations with (inter)national partners and (inter)national funding agencies. Together, this highlights our work as high standard and scientifically relevant.

On January 1st, 2022, BPRC housed 985 monkeys, 638 rhesus macaques (*Macaca mulatta*), 240 cynomolgus monkeys (long-tailed macaques; *Macaca fascicularis*) and 107 common marmosets (*Callithrix jacchus*). On December 31st, 2022, BPRC housed 944 animals, 617 rhesus macaques, 230 long-tailed macaques and 97 common marmosets. In 2022 BPRC worked with 180 animals, 143 rhesus macaques, 32 long-tailed macaques and 5 common marmosets. These numbers were reported to the NVWA.

BPRC is committed to health research and alternatives. The development and implementation of the 3Rs, **R**efinement, **R**eduction and **R**eplacement are visible throughout BPRC. In this report you will find many examples of how refinement of animal models leads to a reduction of the number of animals we work with.

Why do we still need animals for research?

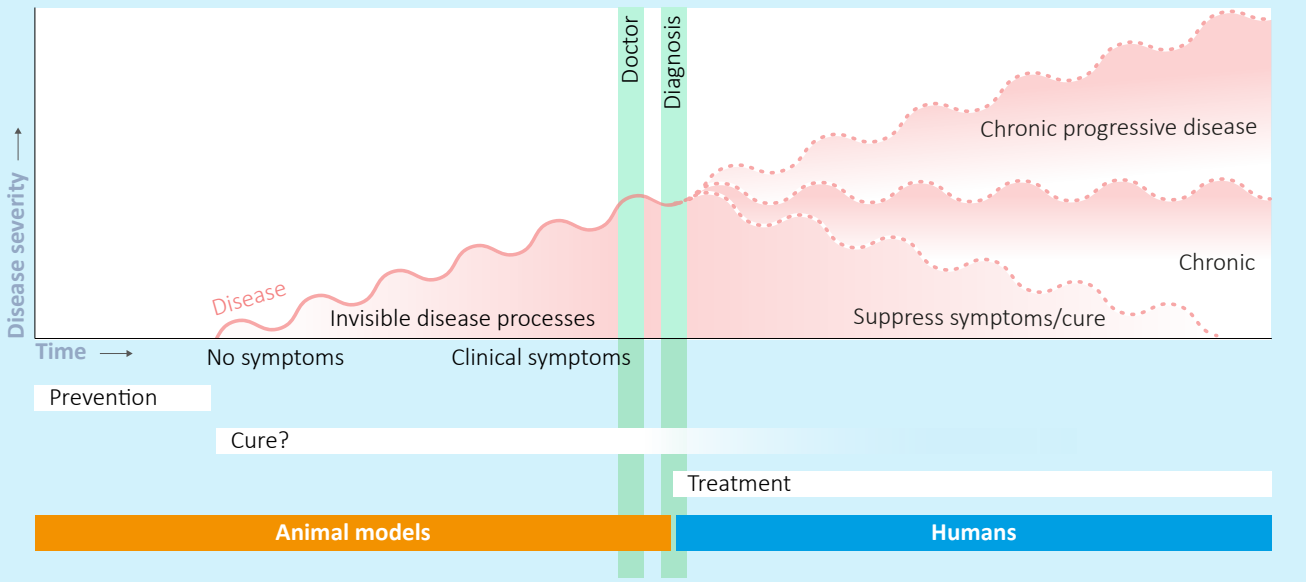
BPRC focuses on life threatening and/or debilitating diseases that affect millions of people. Diseases without cure or treatment because the complicated disease mechanisms are not yet fully understood. The European Commission concluded that the type of research conducted at BPRC cannot be done without life animals.

Visualizing invisible disease processes

A patient only seeks medical help when he or she is suffering from disease symptoms. At that time the actual disease-process is already ongoing and caused damage to cells and/or organs. As a consequence, early and asymptomatic stages of a disease cannot be studied in people. To ‘visualize invisible’ disease processes we depend on experimental animal models.

Monkey Research

- Explained -



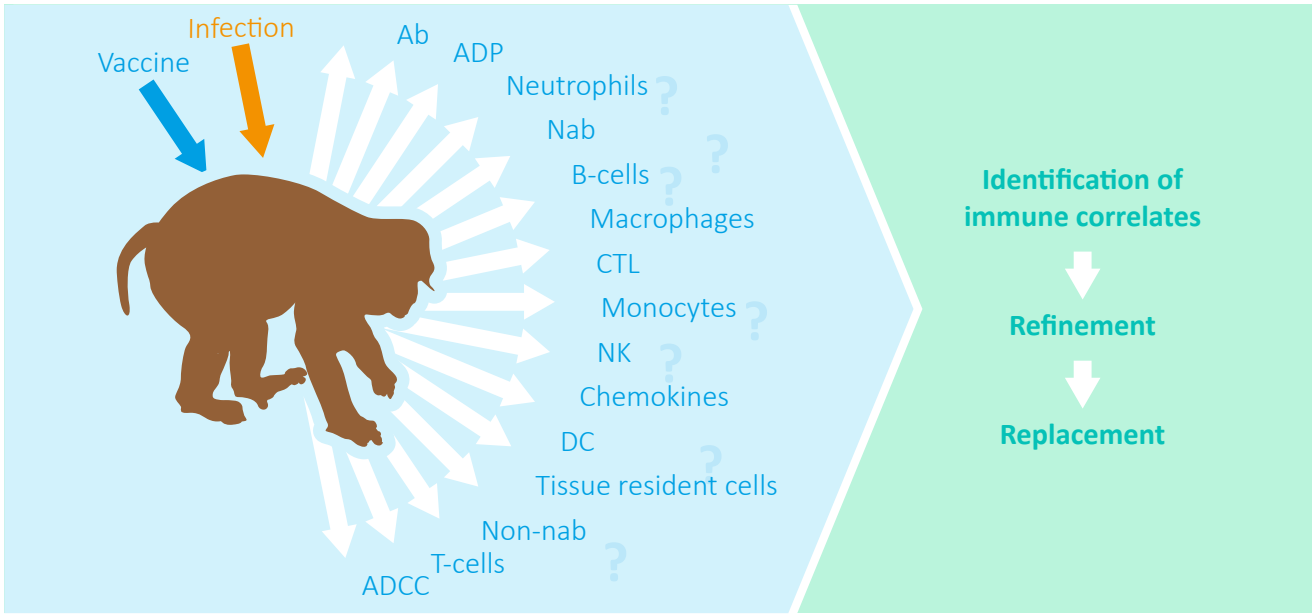
The X-axis represents the time and y-axis disease severity. A disease does typically not start with clinical symptoms. The onset is often without signs. But as time progresses the damage to cells and organs accumulate and cause clinically relevant disease. Depending on the disease this can take days to years. At that point patients go to a doctor and laboratory tests are needed to make a diagnosis. Only when diagnosis is made, proper treatment can start. Most diseases have a so-called point of no return. Before that the damage can be repaired, but when damage progresses beyond that point it results in irreversible (unrepairable) damage.
In best case, the disease is diagnosed before the point of no return. Damage caused by the disease is reversible and treatment cures the disease. In case of a chronic disease, the disease cannot be cured. Drugs can help to suppress further disease progression but the damage is irreversible and drugs cannot undo the damage (MS). During a chronic progressive disease, the symptoms are also irreversible and get worse. So far there are no drugs available to stop progression. Some infectious diseases are preventable by a vaccine (measles) or prophylactic drugs (malaria). Studying a disease and potential new medicines in people is only possible after the diagnosis of the disease. To study early events, we rely on animal models that resemble the infection or disease in humans. Understanding early events of a disease enables the identifying of the point of no return, hence decrease overall medical health care costs and increase quality of life. But also, to develop animal-free alternative methods to evaluate new potential medicines.

Creating conditions for animal free alternatives

Unraveling disease processes is not only necessary to identify potential treatments but also to create the conditions for animal free-alternative methods to test vaccines or new treatments. Before one can even think about the development of an animal-free method to evaluate potential drugs or treatment, one needs full understanding of a disease and its critical events.

Monkey Research

- Explained -



In a prophylactic vaccine study, a vaccine is used to generate a pathogen-specific immune response. The interplay between thousands of different molecules, including antibodies, cytokines, specific subsets of cells in the blood and chemokines, determine the quality and quantity of the immune response, and thus the protective effect of the vaccine. To test this, the animal is exposed the actual pathogen. The protective capacity of the vaccine is defined by the amount of virus, bacteria or parasite that can be detected after exposure. Little or no pathogen means the vaccine was successful.

Identification of (a combination of) molecule(s) that predict the effectiveness of the vaccine on forehand is a powerful refinement of an animal model. In the first place because evaluation of future new vaccine candidates does no longer require exposure to the pathogen itself to determine the effectiveness of a vaccine and therefore the discomfort of the animal is reduced. And second because it is the first step to the development of animal-free alternative techniques to evaluate potential new vaccine candidates.

Vaccine efficacy studies

Vaccines are a safe way to generate immune memory without the potential risk to develop disease-associated complications. Many infectious diseases can be prevented by vaccines, but for many pathogens vaccines are desperately needed. To evaluate the efficacy an experimental vaccine so called exposure studies are required. After vaccination the immune response is challenged by the actual pathogen. This requires a model that is susceptible to vaccination and the pathogen. This makes rodents often not the best model.

Monkey Research

- Explained -

As most vaccines evaluated at BPRC are developed for human use, people would be the best model. However, only for a limited number of infectious diseases human challenge studies are permitted, like malaria and influenza. To limit medical risk for the human volunteers, these human exposure studies are typically performed with weak, attenuated or curable strains of the pathogens, and only with vaccine candidates that have proven safety in animal models. Yet for the vast majority of the vaccine efficacy studies conducted at BPRC human challenge models are not available.

In this report we proudly present our contribution to science, the 3Rs and the development of animal-free alternatives.

External links:

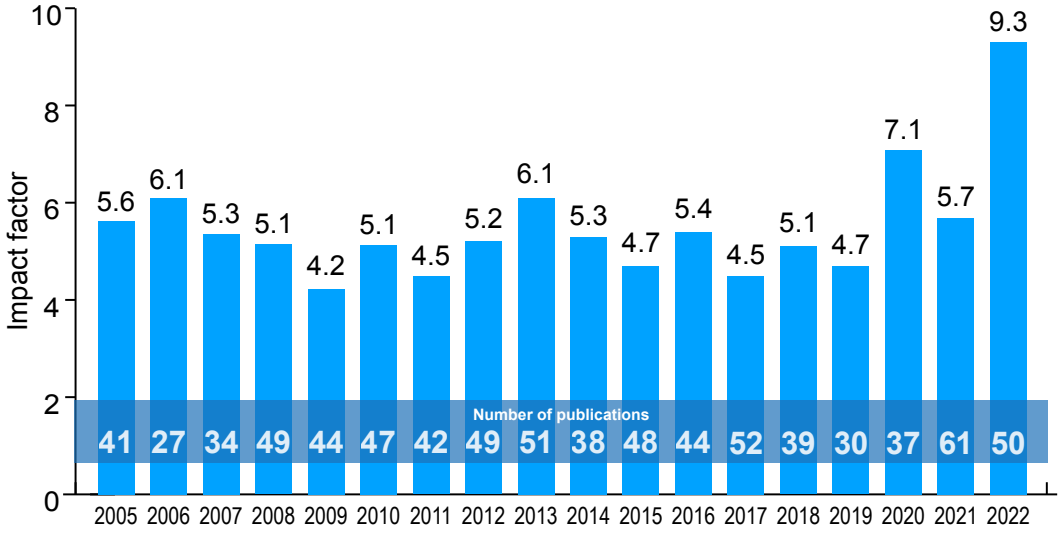
More information regarding animals in experiments is found at Stichting Informatie Dierproeven, European regulatory bodies (Directive 2010/63/EU), the Dutch law (Wet op de dierproeven). The Centrale Commissie Dierproeven (CCD) is the legal body in The Netherlands that is authorized to provide licenses. BPRC's accreditation by AAALAC International guarantees good institutional policies, animal husbandry and welfare, veterinary care at BPRC.

Message from the board

For BPRC and its staff, the year 2022 was, seen from a scientific point of view, very productive. We continued our research on various COVID-19 vaccine candidates but had more time to focus on other biomedical relevant topics as well.

All the different departments have summarized their major achievement on another section of this Annual Report and a comprehensive overview is listed on the news item section on our webpage.

Altogether we published 50 manuscripts in the peer reviewed scientific literature with an average impact factor of 9.3.

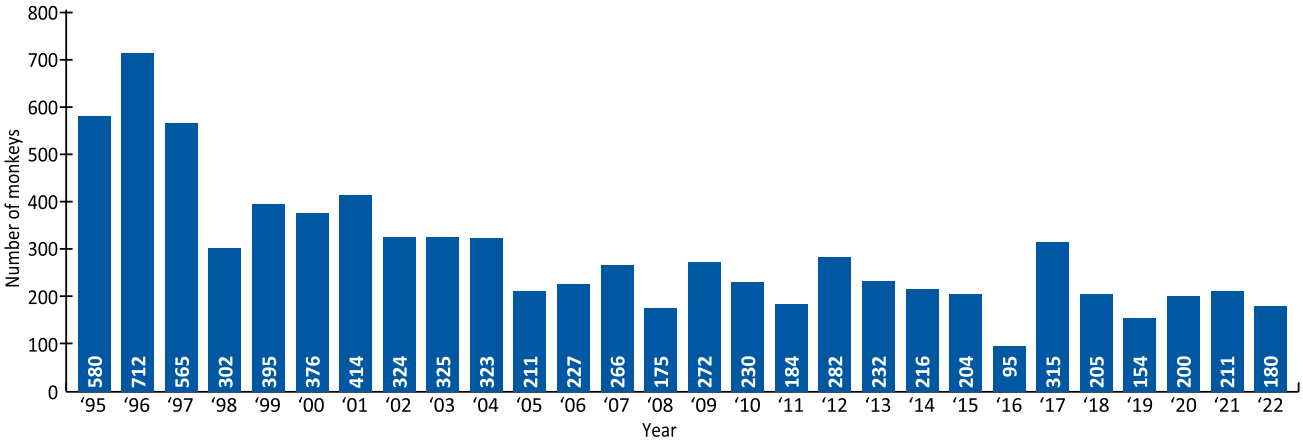


This high number is due to the fact that we published a substantial segment of our research output in leading scientific journals. We consider this as a major achievement and are very proud that improved our publication standards.

Message from the board

On top of that, one of our postdocs, Jesse Bruijnesteijn, received the Julia Bodmer young investigator award at the EFI meeting which was held in Amsterdam.

This year was due to all the hard work financially sound and we closed the books with a small negative result of 8000 euro.



Prof. dr. R.E. Bontrop
Director of BPRC

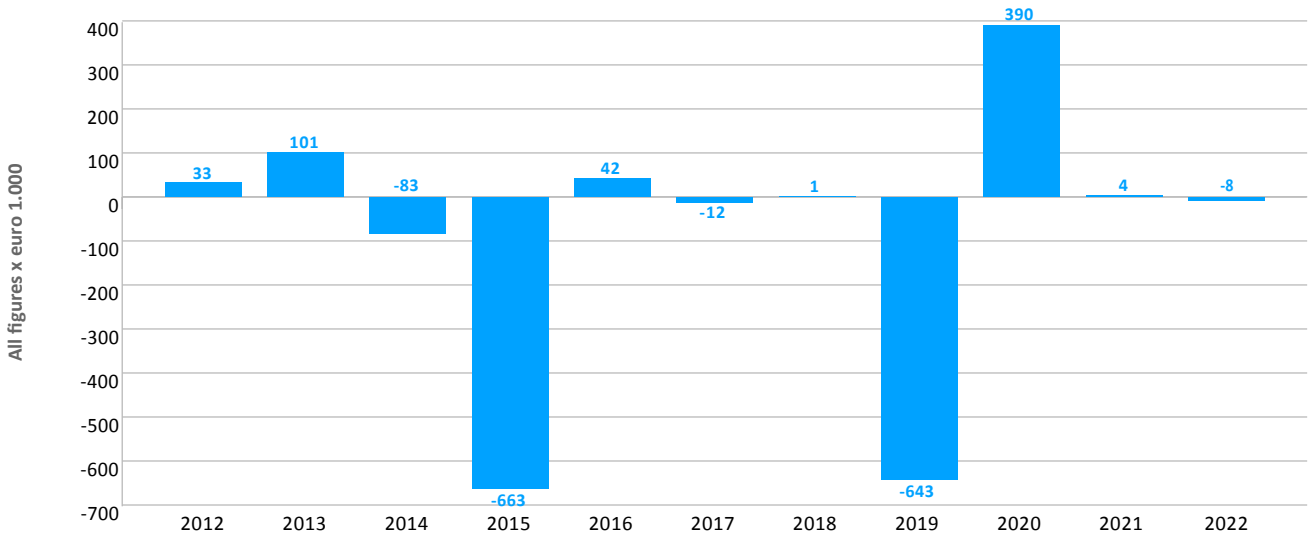
Our Financial Results

Foundation Biomedical Primate Research Centre (BPRC) closed the fiscal year 2022 with a negative result of 8 K€.

Total turnover projects decreased from 13,9 million euros in 2021 to 13,2 million euros in 2022, a difference of 0,7 million euros. The decrease in income commissioned by third parties is caused by the TB Vaccin project which did not start in 2022 but will start in 2023.

The operational costs are three percent lower than the budget for the year 2022.

Result development BPRC 2012-2022



Our Financial Results

	2022 (K€)	2021 (K€)
Turnover projects (extern)	4.280	5.174
Turnover projects (subsidy)	8.747	8.375
Total turnover projects	13.027	13.549
Other excluding interest	211	353
	211	353
Total turnover	13.238	13.902
External direct project costs	346	578
Staff costs	8.095	8.276
Depreciation	589	742
Other operating charges	4.177	4.237
Total operating costs	13.207	13.833
Profit/loss on ordinary activities	31	69
Interest	39-	65-
Profit for the financial year	8-	4
Tax	-	-
Profit for the financial year after tax	8-	4

EFFECTIVE PERSONNEL

	2022		2021	
Service Departments	16,3	17%	15,8	15%
Animal Sience Department	43,5	44%	44,2	43%
Research	38,9	39%	43,2	42%
Total	98,7	100%	103,2	100%

	2022 (K€)		2021 (K€)	
ASSETS				
FIXED ASSETS				
Buildings and structures	24.106		25.342	
Tangible fixed assets	1.306		1.603	
		25.412		26.945
CURRENT ASSETS				
STOCKS		65		72
DEBTORS DUE WITHIN ONE YEAR				
Work in progress	3.179		1.752	
Receivables from contracts	620		973	
Receivables tax	39		17	
Other receivables	196		235	
		4.034		2.977
Cash at bank and in hand		11.975		13.294
Total assets		41.486		43.288

	2022 (K€)		2021 (K€)	
LIABILITIES				
EQUITY				
Equity	4.513		4.508	
Revaluation reserve buildings	4.869		5.388	
Result current year	8-		4	
		9.374		9.900
PROVISIONS				
Primates	-		-	
Deferred tax liabilities	-		-	
(Flexibel) retirement	-		-	
Repairs buidings	1.381		1.910	
		1.381		1.910
LONG TERM DEBTS				
Bank	17.904		19.099	
Received in advance on asstes	5.901		6.034	
		23.805		25.133
SHORT TERM DEBTS				
Received in advance on projects	2.637		1.609	
Received in advance on assets	311		369	
Received in advance subsidy	740		700	
Accounts Payable (TAX)	404		456	
(Flexibel) retirement	104		103	
Accounts Payable	620		789	
Commitment Bank	1.195		1.138	
Other liabilities	915		1.181	
		6.926		6.345
Total liabilities		41.486		43.288

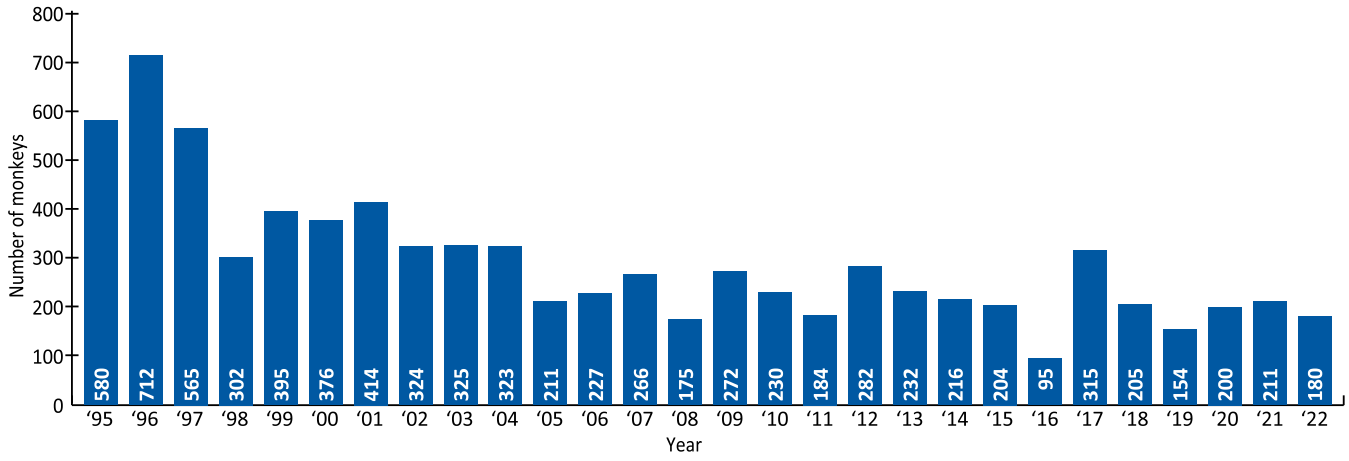
Our Financial Results

WNT-Verantwoording Organisatie BPRC	
BEZOLDIGING TOPFUNCTIONARISSEN	
Leidinggevende topfunctionarissen	
Gegevens 2022	
Bedragen X 1€	
Functie(s)	Directeur
Aanvang en einde functievervulling in 2022	1/1 - 31/12
Omvang dienstverband (in fte)	1,0
Dienstbetrekking	Ja
Bezoldiging	
Beloning plus belastbare onkostenvergoedingen	166.174
Beloningen betaalbaar op termijn	28.886
Subtotaal	195.060
Individueel toepasselijk bezoldigingsmaximum	216.000
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT
Bezoldiging	195.060
Reden waarom de overschrijding al dan niet is toegestaan	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT
Gegevens 2021	
Aanvang en einde functievervulling in 2021	1/1 - 31/12
Omvang dienstverband (in fte)	1,0
Dienstbetrekking	Ja
Bezoldiging	
Beloning plus belastbare onkostenvergoedingen	162.096
Beloningen betaalbaar op termijn	31.217
Subtotaal	193.313
Individueel toepasselijk bezoldigingsmaximum	209.000
Bezoldiging	193.313

Toezichthoudende topfucntionarissen					
Gegevens 2022					
Bedragen X 1€					
Functie(s)	Lid Raad van Toezicht (Voorzitter)	Lid Raad van Toezicht (Voorzitter)	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht
Aanvang en einde functievervulling in 2022	1/1 - 30/06	1/7 - 31/12	1/1 - 30/06	1/1 - 31/12	1/12 - 31/12
Bezoldiging					
Bezoldiging	4.639	4.639	3.328	6.959	0
Individueel toepasselijk bezoldigingsmaximum	32.400	32.400	21.600	21.600	21.600
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT	NVT	NVT	NVT	NVT
Reden waarom de overschrijding al dan niet is toegestaan	NVT	NVT	NVT	NVT	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT	NVT	NVT	NVT	NVT
Gegevens 2021					
Aanvang en einde functievervulling in 2021	1/1 - 31/12		1/1 - 31/12	1/1 - 31/12	
Bezoldiging	9.278	0	6.655	6.959	
Individueel toepasselijk bezoldigingsmaximum	31.350	0	20.900	20.900	

Gegevens 2022				
Bedragen X 1€				
Functie(s)	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht
Aanvang en einde functievervulling in 2022	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging				
Bezoldiging	6.959	6.655	6.959	6.959
Individueel toepasselijk bezoldigingsmaximum	21.600	21.600	21.600	21.600
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT	NVT	NVT	NVT
Reden waarom de overschrijding al dan niet is toegestaan	NVT	NVT	NVT	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT	NVT	NVT	NVT
Gegevens 2021				
Aanvang en einde functievervulling in 2021	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging	6.959	6.655	6.655	6.959
Individueel toepasselijk bezoldigingsmaximum	20.900	20.900	20.900	20.900

Facts & figures



The number of monkeys we worked with over the years and the scientific achievements in 2022.

A total of

50 scientific publications

in peer reviewed international journals.
Complete list

In addition we

train eight PhD students

to write and defend their thesis.

We placed

19 updates on our website

in the newsfeed.

Health, Safety & Environment

Health and Safety of our colonies and our staff as well as the care for our environment is part of BPRC's policies. We have an environmental management system which is ISO14001 certified (ISO14001:2015) and through which we manage and continuously try to reduce our environmental footprint.

Key aspects of BPRC's Health, Safety and Environmental policy are:

- Microbiological safety; preventing employees from being infected
- Protecting the environment from the release of biological agents (including GMOs)
- Restricting the use of hazardous substances
- Efficient energy and water consumption.

We consider the following aspects integral components of our operations:

- Continually raising awareness of safety and environmental concerns and providing information on these concerns to all employees and guests
- Appointing employees tasked with carrying out the duties arising from our Occupational Health and Safety and Environmental Protection Policies
- Informing employees and others of the quality
- Creating the right facilities and ensuring that installations and equipment are used properly
- Identifying, recording and properly addressing complaints regarding health-and-safety and environmental protection issues
- Having annual internal audits performed.

Health, Safety & Environment

Energy consumption

In recent years BPRC invested in the reduction of energy consumption, for example by replacing light sources for more efficient LED-lights, an effort that has continued in 2022.

Safety awareness & training

Safety awareness is an essential part of the daily routine and BPRC staff is periodically trained in all aspects of safety. For example; a training for radiological safety has been organized for all personnel that work with, or in the vicinity of, ionizing radiation which is part of the experiments involving our PET-CT.

Also, an awareness training for chemical safety was organized for all personnel that frequently uses chemicals on their workplace.

BPRC's Research Areas

Modeling potentially life-threatening human diseases in non-human primates requires extended knowledge and dedication in animal care taking, colony management and translational research. Scientists from BPRC are world-wide acknowledged for their expertise in the translation of human diseases caused by viruses and parasites to non-human primates, as well as autoimmune diseases and genetics.

Research Areas
Alternatives

Monkeys are similar to humans. Not only on the outside but also on the inside.
That is because monkeys are genetically related to us. Due to this evolutionary relationship monkeys sometimes are a good model to study human diseases. But only if there is no other way.

Working with monkeys brings a great responsibility. We are responsible for the well-being of the animals in our colonies. We continuously seek to conduct research that does not involve animal testing in order to reduce the numbers of animals we work with. In the meantime, we accommodate and look after our monkeys with the best possible care.

We do this using the principles of the 3Rs. Refinement, reduction and replacement. Refinement and reduction go hand in hand as Refinement of an animal model will lead to a Reduction of the number of animals per experimental group.

3Rs throughout BPRC

Refinement

- Improvement of animal welfare is a continuous process in our institute. BPRC staff take part in (inter)national training programs to remain their high standards and gain new insights.
- All animals are socially housed.
- Stress is not good. It affects animals in breeding groups and can even affect the results of an experiment. In order to avoid stress you need to identify stressful events. And for that you need unbiased, objective and reliable parameters to determine stress.
    - Measuring the cortisol levels in hair samples is a method that can provide stress information from an individual animal. By cutting a hair into smaller pieces you can relate the cortisol levels to potential stressful events.

Research Areas
Alternatives

  - We take pictures as an objective measure for alopecia. Alopecia (hairless body parts) can be a sign for acute stress. Caretakers are trained to detect this and to take pictures. Sometimes an animal experiences stress from hierarchy in their breeding group. If that is the case behavioral scientists are notified to monitor the breeding group and if possible take measures.
  - When animals are prepared for housing in an experimental setting they are introduced to a selected cagemate. We can use round the clock camera recordings to monitor their behavior in the absence of a caretaker. This avoids less-compatible pair-housed animals.
- Positive reinforcement training (PRT). We have trained 25 animal caretakers how to train their animals. They do this twice per week. With this training method we are able to perform certain biotechnical techniques without sedating the animal.
- All marmosets jump voluntarily on a scale. This way their body weight can be monitored without sedation.
- All experimentally housed animals were trained to drink from a syringe, thus voluntarily take oral medication.
- Caretakers spent 15% of their time on (cage)-enrichment. For instance assembling food-puzzels, providing animals with toys or redecorate enclosures.
- Further improvements were implemented in diet variation, to maximize natural feeding routines.
- In 2017 an improved version of the ‘Welzijnsevaluaties’ was implemented.
- New features were introduced in our monkey database for the daily registration of each individual animal.
- All animals in experiments are observed at least twice a day. During this observation different parameters are ‘scored’. Normally an animal shows a broad variety of natural behaviors. In some models for (infectious) diseases the animal’s behavior changes. This is however a subjective parameter and changes are difficult to observe. Subtle changes during an experiment can provide crucial information. In this case we prefer to measure physical activity with telemetry. These devices register X-Y-Z coordinates of individual animals. If necessary it is also possible to measure body temperature, heartrate, blood pressure. This will lead to further refinement of our animal models.

Research Areas
Alternatives

Reduction

Optimizing and standardizing in vitro laboratory tests play an important role in the reduction of the animals we work with. Also in 2018 we have implemented new techniques. By using these new conditions, we aim at less variation in laboratory tests that will lead to smaller group sizes in our animal experiments.

Genetics

Genes play an important role in infections and diseases. We have implemented new techniques to determine the genetic background of animals in the breeding and experimental colonies. This enables us to select (or deselect) appropriate animals to answer particular research questions. For example; we know that certain genes play a role in the development of AIDS after HIV infection. We now know that these genes are also present in monkeys. Selection of animals for an HIV experiment is therefore based on these genes. Proper selection reduces the variation in an experiment and therefore smaller group sizes are required to obtain statistical significant differences.

Statistics at BPRC

One of the hallmarks of good science is statistics. Not only at the end of a proof on concept study to determine whether an HIV-vaccine was successful but also during the design of the study. Therefore, good statistics is part of the 3Rs.

Statistics is often used to determine whether differences in study outcomes are (statistically) significant. This is normally done by rejecting or accepting the null hypothesis, where the null hypothesis states that treatment does not have a significant effect. To do so, the p-value is calculated. If the p-value is below 0.05, the chance that the study outcome arose by chance is smaller than 1 in 20. In that case, the null hypothesis is rejected, supporting the alternative hypothesis that the observed difference was due to the treatment.

But statistical testing is only informative if the study is properly designed. If group sizes are too small a real difference may not be detected and the study will not be informative. If group sizes are large differences will be detected, but at the cost of too many animals. Therefore study design involves, amongst other things, also a so called “power calculation”.

Research Areas
Alternatives

The number of animals per group is calculated based on the desired effect of the treatment on the primary outcome (e.g. diseased or not-diseased), the between-animal variation of the treatment effect and the desired power. The desired power is the chance that a real difference, if present, is detected. This is usually set at 80% (i.e. 80 out of 100 studies will yield significant results). Next to the power calculation, the study design also involves methodological topics like randomization of the animals (treatments are allocated by chance) and blinding of observers (treatment is not known). Next to the power analysis, a statistical analysis plan is written before the study is performed. Because monkey studies are often the last step before testing in humans, monkey studies should be designed, performed, analyzed and reported in a similar fashion as clinical trials in humans.

PET-CT
ositron emission tomography–computed tomography (PET-CT) is a visualization technique that combines anatomic localization (X-ray) and functional imaging (nuclear medicine). In hospitals, PET-CT is already widely used during the diagnosis and treatment of cancer. Over the last years, PET-CT also proved its additional value to biomedical research with animals.

PET-CT offers many advantages over traditional techniques. First, PET-CT is minimal-invasive. Second, as results from blood tests, biopsies/swabs or cells washed out of the organ of interest can be indicative for infection, they are often poor indicators for actual disease manifestations. Besides biopsies only provide information of the tissue in the biopsy but often not of the entire organ. The combination of X-ray and specific radioactive probes allows screening of the entire body in both an anatomical and functional way. This minimizes the discomfort of the animals and provides you a much broader view.

In addition, PET-CT offers the opportunity to visualize disease progression or therapeutic response over time (longitudinal). This is particularly relevant when critical organs need to be studied, like lungs or brains. PET-CT in combination with 18F-Fluorodeoxyglucose (FDG) as imaging agent is well-established used for about 90% of the PET-CTs obtained in human. FDG visualizes the glucose metabolism in the body and shows increased signal in areas with

Research Areas
Alternatives

an increased metabolic activity. Increased metabolic activity can be due to cancer, infection/ inflammation though also after a surgery in the area where a scar is healing. This makes FDG PET-CT highly sensitive for detecting for instance tuberculosis and influenza in the lungs.

BPRC already started to use PET-CT in 2017. Initially only in our tuberculosis research but currently we are applying this state-of-the-art technique also in other research programs like influenza. With this, PET-CT is not only leading to new and more extensive scientific insights though also increasing the translational value of our animal models as PET-CT is a well-accepted imaging method in humans.

Replacement

In 2009 BPRC-researchers developed an new in vitro assay to test drugs for it's anti-malaria activity. This assay replaces the use of monkeys. Last year we tested 33 new potential anti-malaria drugs with this assay. Before 2009, 33 monkeys would have been necessary to test these 34 compounds. So far, BPRC tested 999 drugs with the animal-free assay.

Research Areas
Alternatives

3Rs Alternatives Unit BPRC

At BPRC, we are fully aware of our responsibility to animals and society. We use animals for our research only to study essential questions around serious and life-threatening diseases for humans and when these questions cannot be answered with non-animal methods or with animals other than monkeys.

Alternative methods are categorized along the principle of the 3Rs of Replacement Reduction and Refinement, all of which have a place within BPRC. BPRC is committed to the implementation of the 3Rs and is actively involved in the development and implementation of 3Rs methods. The 3Rs are implemented in the research of every department.

BPRC is currently restructuring the Alternatives department to be able to respond even better to developments in the field of alternatives and implementation of the latest techniques. Recently, a new Head of Department has been appointed. She will be the connecting person for the different activities of the various departments regarding alternatives and be actively involved in the development and implementation of new methods in this rapidly developing research field.

Research Areas
Ethology

Monkeys are social animals that both compete and cooperate with their group members. Understanding the dynamics in behaviour of monkeys is not only important for scientific behaviour research, but also to manage our breeding and experimental colonies.
To improve our knowledge, we work together with a group of behavioural scientists from the University of Utrecht.

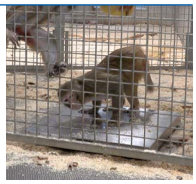
Living in natural groups enhances both welfare and female reproduction

It is currently common practice to house non-human primates at biomedical research facilities, e.g., rhesus macaques, in social groups. To enhance female reproductive success, peer groups are formed. In these breeding groups, infants are taken from their mother at an age of ten months and housed with animals of approximately the same age. Yet for welfare, leaving offspring with their mother and allowing multigenerational groups including families is preferred. This argues that a trade-off between female reproductive success and welfare exists. In this retrospective study we investigated the differences in female rhesus macaque reproductive success between peer groups and multigenerational groups. Our results show that females in multigenerational groups have more births per year and have higher offspring survival compared with those in peer groups. Thus, housing rhesus macaques in multigenerational groups provides a win-win situation, rather than a trade-off, in which female reproductive success and animal welfare can simultaneously be optimized.

Read more >

Research Areas
Ethology

Photo: Animal Behaviour and Cognition, Utrecht University



Monkey weight watching

Captive monkeys have an easier life than their wild counterparts: they have abundant food and do not have to work hard to get it. This can lead to unwanted overweight. We explored whether overweight can be predicted and contained.

Individual variation in non-human primate adiposity may result from differences in behaviour related to energy intake, energy expenditure and dominance rank.
We investigated whether behaviour predicts weight gain during adulthood in captive female rhesus macaques. At baseline, overweight was associated with low activity levels, but not related to food intake, age or dominance rank. In contrast, the increase in overweight after two years was not related to baseline food intake and activity budgets, while higher-ranking females had a higher increase in weight compared to lower-ranking monkeys. This suggests that captive monkeys with a high dominance rank are more prone to becoming overweight, whereas differences in activity budgets are merely a consequence and not a cause.
This suggests that increasing monkey activity levels will not lead to less overweight.
Overweight, however may be contained by controlling the amount of food provided.
This has been applied to individually- and pair-housed monkeys, but whether this can be effective and safe in group-housed monkeys has not yet been assessed.

Research Areas
Ethology

This non-invasive study investigates the effect of a mild reduction of the total amount of food on adult overweight and cholesterol, and on immature growth, veterinary consultations, and reproductive success in multigenerational long-tailed macaque breeding groups. After a period with a reduced amount of food, heavier individuals and females lost more weight compared to leaner individuals and males. Cholesterol levels became lower in adults. Immature growth, veterinary consultations and female reproductive success were not affected. Altogether, providing less food in particular targeted overweight adults, and had no adverse effects on the variables examined in this study. This implies that controlling the amount of food can be a valuable overweight management strategy in group-housed monkeys.

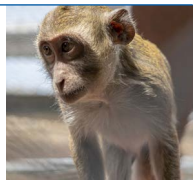
Understanding how behaviour and feeding regimes contribute to becoming overweight provides opportunities to improve housing and husbandry of captive group-living monkeys.

Does behaviour predict weight gain during adulthood in captive group-living rhesus macaques?
Read more >

Overweight Management through Mild Caloric Restriction in Multigenerational Long-Tailed Macaque Breeding Groups
Read more >

Research Areas
Ethology

The older the better: older macaque males are nicer to infants



In captive primates, new males have to be introduced regularly to prevent inbreeding. Sometimes, these males commit infanticide, i.e., the killing of young infants.
More knowledge of the risk factors that are associated with infanticide may lower the incidence of infanticide during male introductions. We used explanations of infanticide from wild data and the anti-infanticidal strategies of females to predict these risk factors. Next, we tested these factors using demographic data collected on captive long-tailed macaques over a long period. The ages of both infants and new alpha males are important: infants under the age of 215 days are at risk of being killed, and typically young males (8 years of age) commit infanticide. Therefore, to lower the risk of infanticide during male introductions in captivity, we advise introducing only males in their prime age (9 years of age), preferably in periods with no infants younger than 215 days of age.

Read more >

Research Areas

General Primate Biology & Welfare

Maintaining the health and stability of the monkeys in our self-sustainable breeding colonies and research facility requires dedication and expertise. This is a joint effort between animal caretakers, veterinarian staff, ethologists, laboratory staff and experts in genetics.

Colony Management and birth control

Contraception is used for management purposes in our group housed non-human primate colonies. Frequent individual oral treatment is challenging and unreliable in socially housed macaques whereas surgical intervention is reliable but requires an invasive intervention and is in principle irreversible. Therefore, long-acting reversible contraceptives are used as they eliminate logistical problems associated with daily, weekly, or monthly administration. Etonogestrel (ENG) implants are progesterone only releasing rods that are reversible, long acting for at least three years, and commercially available for human as Impanon© or Nexplanon©. We have performed a retrospective data analysis detailing the contraceptive effectivity, dose and reversibility of subdermal inserted ENG implants. A success rate of 99.8% and 99.95% with ENG was observed for rhesus- and cynomolgus macaques respectively. ENG had no clinical effect on hemoglobin and blood chemistry parameters nor on the thickness of the endometrial lining or uterus volume. In 2023 more detailed analyses are performed.

Research Areas

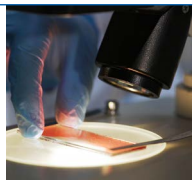
General Primate Biology & Welfare

Environmental data in the breeding colony housing

In collaboration with expert groups on air quality from the Veterinary Faculty of Utrecht University the indoor environment of the breeding groups was assessed.
The indoor temperature and relative humidity for both species were within comfortable ranges. The geometric mean (GM) ammonia, dust and endotoxin concentrations were within accepted human threshold limit values. The GM dust concentrations were significantly higher during the daytime than during the nighttime. Care must be taken that levels do not exceed accepted threshold values.

Read more >

Blood parameters



A retrospective longitudinal cohort study to estimate reference intervals for hematologic and serum biochemical values in clinically healthy macaques based on observed percentiles without parametric assumptions was conducted. In total, 4009 blood samples from 1475 macaques were analyzed with a maximum of one repeat per year per animal. Data were established by species, gender, age, weight-for-height indices, pregnancy, sedation protocol, and housing conditions. Most of the parameters profoundly affected just some hematologic and serum biochemical values. The results emphasize the importance of establishing uniform experimental groups with validated animal husbandry and housing conditions to improve the reproducibility of the experiments.

Read more >

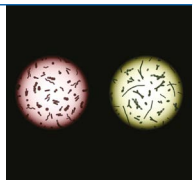
Research Areas

General Primate Biology & Welfare

Evaluation of antibiotic use

It is common practice to use antibiotics off-label to treat bacterial infections in macaques (Macaca sp.) housed in zoos, sanctuaries and in biomedical research centers. This implicates a lack of scientifically justified proof regarding efficacy and dosing of most of these drugs. Antibiotics with long elimination half-lives (>48h) and therefore requiring less frequent administration (long-acting antibiotics) would be very useful to treat macaques, as this will reduce handling related stress. Therefore, the pharmacokinetics (PK) of various antibiotics, such as the long-acting ampicillin (AMP-LA), is assessed in macaques. The first results of this ongoing research will be available in 2023.

Microbiome Research



The mammalian virome (the total collection of viruses in and on the organism) has been linked to health and disease but our understanding of how it is structured along the longitudinal axis of the mammalian gastrointestinal tract (GIT) and other organs is limited. We have been involved in the metagenomic analysis of the prokaryotic and eukaryotic virome occupying various parts of the GIT, as well as liver, lung and spleen, in rhesus macaques. The data show that GIT virome composition was specific to the anatomical region. Upper GIT and mucosa-specific viruses were greatly under-represented in distal colon samples (representative for faeces). Nonetheless, certain viral and phage (viruses infecting bacteria) species were found ubiquitously in all samples from the mouth to the end of the colon. The dataset and its accompanying methodology may provide an important resource for future work investigating the biogeography of the mammalian gut virome. Currently, we are studying the microbiome in healthy and diseased macaques.

Read more >

Research Areas

General Primate Biology & Welfare

Observations and training of animals

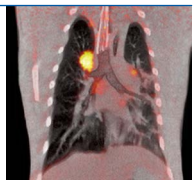
Behavioural observations and training of animals are very important topis at the BPRC. More than 60 cameras are currently installed, both in the breeding and in the experimental facilities, to observe behaviour of the animals when no humans are present. Knowledge of the behaviour of animals in the absence of humans is important to assess if animals are behaving normally. Positive Reinforcement training is an important topic at the BPRC and is widely implemented.

Research Areas

Medical imaging

The aim of experimental animal models is to understand infection and disease to enable effective prevention or treatment. Our studies in NHPs include research on various physiological and anatomical aspects of diseases, such as infectious diseases, neurological and aging-related diseases, including pathogen dynamics, immune responses, and host pathology. To study these various aspects, non-invasive imaging techniques, such as Chest X-ray (CXR), computed tomography (CT), and positron emission tomography - computed tomography (PET-CT) are applied.

Medical imaging in SARS-CoV-2 infections



Since the outbreak of the coronavirus disease 2019 (COVID-19) multiple studies have been performed to understand, control, and halt the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In these studies, the role of medical imaging to assess the pulmonary disease has been investigated to complement the virological measurements and to obtain meaningful translational readouts of lung disease. A review of the literature within the Bill & Melinda Gates Foundation consortium regarding SARS-CoV-2 imaging in non-human primates indicates that medical imaging of SARS-CoV-2-exposed NHPs enables high-resolution qualitative and quantitative characterisation of disease otherwise clinically invisible and potentially provides user-independent and unbiased evaluation of medical countermeasures.

Read more >

Research Areas

Medical imaging

Novel tracers to study inflammatory responses during SARS-CoV-2 infection

Within the BPRC we investigated this even further with the application of new radiotracers more focused and dedicated for the visualization of inflammatory processes. One of these targets is the mitochondrial translocator-protein (TSPO). TSPO is widely distributed over the entire body but is upregulated in activated microglia and systemic monocytes. Until now its application has been mainly limited to imaging of neuroinflammatory purposes but as TSPO is distributed over the entire body it may be possible to visualize inflammatory processes in other organs as well.

We illustrated with this study that this was true, [18F]DPA714 with TSPO as target is a valuable radiotracer to visualize SARS-CoV-2-associated pulmonary inflammation, which coincided with activation of dendritic cells in blood. [18F]DPA714 thus has the potential to be of added value as diagnostic tracer for other viral respiratory infections.

Read more >

Research Areas
Tuberculosis

Tuberculosis (TB) is a bacterial infection that causes lung disease. TB is still one of the deadliest infectious diseases by a single pathogen in the world. Progress made in the years up to 2019 has slowed, stalled, or reversed, and global TB targets are off track (WHO report 2022). For the first time in over a decade TB death increased in 2022 to 1.6 million. Only to be surpassed by the number of people who died of COVID (3.55 million in 2021). COVID 19 has severely impacted on access to essential services put in place to fight TB. Fewer people were diagnosed and treated or provided with TB preventive treatment and fewer resources (*funding*) for essential TB services and R&D were available. In addition, we are still confronted with the fact that approximately 25% of the world population is latently infected with TB.

Antibiotics are currently the only treatment options in bringing the disease down. However, anti-microbial drug resistance in TB is on the rise, which makes it harder to treat. Overall, a growing number of TB infected people cannot be treated and die of the disease. Prevention by vaccination is important to break the cycle of TB transmission and infection.

Read more >

BPRC continues to use nonhuman primate models of TB, specifically the rhesus monkey and the cynomolgus monkey, to get a better insight in how the disease develops and to evaluate new treatments of TB. TB in these 2 species represents two different disease manifestations. TB in rhesus monkeys develops as a progressive active form of the disease, while infection of cynomolgus monkeys can also develop as a latent disease. Together, these models best recapitulate the range of manifestations of tuberculosis in man.

Research Areas
Tuberculosis

Local administration of BCG-vaccine is superior to classical skin immunization continued

Vaccines aim at the induction of long-lived immune memory specific for the disease you vaccinate against. This memory is mediated by the adaptive immune system consisting of T and B cells. The role of the innate immune system in the context of vaccines is primarily considered to provide the proper help to stimulate the adaptive response. Innate immune responses and function were considered constant and invariable over time. Over the last decade, however, it has become clear that also innate immune cells, like monocytes, macrophages and NK cells can adjust and display memory-like phenotypes. This innate immune 'memory' is now typically referred to as trained immunity. BCG is the prototypical vaccine that, next to a TB-specific effect, can induce trained immunity and provides broad (heterologous) protection against disease from other respiratory infections.

We have previously shown that mucosal vaccination with BCG delivered in the lung provided better protection against TB than classical immunization in the skin and that this protection was associated with unique TB specific local adaptive immune responses.

Read more > article and commentary.

We extended our analyses of the immune response observed after mucosal vaccination with BCG and established that these vaccine-induced T cells in the lung were endowed with increased expression of homing markers and tissue residency markers.

Read more >

Research Areas
Tuberculosis

We showed for the first time in nonhuman primates that we can recapitulate the trained immunity phenotype observed in humans after standard TB vaccination in the skin. In addition, we demonstrated that mucosal vaccination through the lung results in improved trained immunity compared to the standard vaccination.

Read more >

The new vaccine candidate MTBVAC that was derived form a human clinical isolate of mycobacterium and is now in phase III of clinical development, was equally potent in the induction of trained immunity after mucosal vaccination as BCG.

Read more >

To make mucosal delivery in the lung translatable to the clinic we further demonstrated that aerosol delivery, of MTBVAC with a nebulizer, instead of endobronchial instillation, provided robust protection against TB disease compared to non-vaccinated control animals. MTBVAC delivered through aerosol induced comparable immune signatures to what we have seen before upon pulmonary mucosal MTBVAC instillation. (manuscript in preparation).

These results provide support to strategies for improving TB vaccination and prompted us to further investigate whether the mucosal whole cell vaccination could be used for revaccination purposes.

Standard intradermal BCG vaccination is given to millions of children every year to protect them from TB in their childhood. However, at the age of adolescence, the protective efficacy of revaccination standard intradermal BCG against pulmonary TB is highly variable ranging from 0-80%. Altering the route of vaccination in adults (revaccination) might be a way to improve efficacy in these adults.

Research Areas
Tuberculosis

We established that mucosal BCG revaccination after a standard intradermal BCG vaccination resulted in a reduction of TB disease and that this was associated with the same unique local immune signature as was observed with mucosal vaccination in naïve animals (manuscript in preparation).

Research Areas

Comparative Genetics & Refinement

The actions of the immune system are orchestrated by many different genes. The allelic variability of these genes can differ from individual to individual, and this genetic variation is called polymorphism. Polymorphisms explain why some people are susceptible for a certain disease while others may be resistant to development of the same disease. Hence, the diversity generated by genetic polymorphisms warrant unique responses and thus prevent the elimination of an entire population by one single pathogen.

The killer cell immunoglobulin-like receptor (KIR) and leucocyte immunoglobulin-like receptor (LILR) system, which are located in the leucocyte receptor complex (LRC), and the major histocompatibility complex (MHC), are examples of polymorphic immune gene systems. A successful immune response is multifactorial, and depends, for instance, on the cooperation between KIR and MHC. In general, MHC molecules are involved in the presentation of self and non-self peptides to the immune system and thereby facilitate the recognition of invading pathogens, whereas KIR and LILR molecules finetune and serve as correction mechanism (immune checkpoint) for pathogen evasion. KIR molecules are involved in the immune defence to viruses and cancer cells, while LILR molecules play a role as modulators of infection and immunity and are engaged in neural function.

Monkeys are genetically highly similar to humans. Understanding genetic polymorphisms in monkeys and their effect on the immune system, teaches us much about the functional immune defence in humans. This is particularly important in the development of a whole new generation of medicines, the so-called personalized medicines.

In the Comparative Genetics and Refinement department, we investigate *MHC, KIR* and *LILR* gene diversity in different monkey and great ape species. For this, we use DNA-sequencing and other molecular techniques, like fragment analyses on short tandem repeats (STR). We not only study the DNA from animals from our own breeding colonies but also DNA samples from other institutions and zoos.

Research Areas

Comparative Genetics & Refinement

Applying genetics, with a particular focus on the *MHC, KIR* and *LILR* regions, for the refinement of animal models and as tool in conservation biology of non-human primate species

The peak of the COVID-19 pandemic seems to be behind us, but it showed our vulnerability and the widespread impact a pandemic can have on our modern society. To quell this specific pandemic and reopen society, the need for safe vaccines has been high. In that light, the pre-clinical research in monkeys played an important role in the fast development of the variety of COVID-19 vaccines, some of which came available already in 2021.

Worldwide also other infectious diseases like tuberculosis, malaria, flu, and HIV-1 threaten humankind, and demand adequate medical treatments, for instance in the form of new antibiotics and vaccines to prevent the millions of deaths each year caused by these type of infections.

At our institute, preclinical research contributes to the development of therapies and vaccines for a variety of human infectious diseases. In addition, research is applied on animal models of autoimmune diseases, like multiple sclerosis, and disorders that arise during human aging, such as Alzheimer's and Parkinson's disease.

As a genetics department, one of our research goals is the refinement of the different animal models that are subject of study in the institute. The *MHC, KIR* and *LILR* systems encode for molecules that represent important components of the immune system and are known they show a considerable amount of polymorphism. As such, the knowledge that we gained on the diversity of these systems in our animal models is of importance to interpret disease outcome and may aid into translation of therapies and medicines to cure diseases in humans.

Research Areas

Comparative Genetics & Refinement

Long term studies on the characterization of *MHC* diversity in our cynomolgus macaque cohort and the characterization of *KIR* variability in the rhesus macaque population housed at our facilities were both recently finished. These insights can now, for instance, be applied in the selection of animals for specific preclinical studies.

- Dynamic evolution of *MHC* haplotypes in cynomolgus macaques of different geographic origins. Read more >
- Comparative genetics of *KIR* haplotype diversity in humans and rhesus macaques: the balancing act. Read more >

The next generation sequencing technologies that more recently became available, commercialized by Pacific Biosciences and Oxford Nanopore Technologies sequencing platforms, have been successfully implemented in our protocols and have more or less taken over the good-old Sanger sequencing[1-2]. These technologies not only speed up the research that we conduct on the multigene systems, but have also opened up new avenues of research that we can perform. For instance, whole genome sequencing is applied for the characterization of the entire *MHC, KIR* and *LILR* regions. In addition, the platforms allow RNA sequencing of full-length transcripts and enables the in-depth characterisation of other multigene systems to refine our animal models.

The *MHC* and *KIR* typing[1-5], mtDNA analysis[6], and parentage definition by STR analysis are assays we routinely apply to monitor the outbred status of BPRC's breeding groups of rhesus and cynomolgus macaques. This toolkit is also applicable for conservation purposes of non-human primate species housed at other institutions and zoos. The expertise we have developed on ABO typing[7] in monkeys and apes, may provide essential information in case of a blood transfusion. This has resulted in a two-year collaboration with French veterinaries, typing several great-ape species animals, such as chimpanzees, but is also an assay that we occasionally use for the characterization of third-party macaque and baboon species.

Research Areas

Comparative Genetics & Refinement

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Research Areas

Influenza

Influenza (flu) is a contagious respiratory disease caused by influenza viruses. It can cause mild to severe illness. Every year over half a million people die of seasonal influenza. Many different influenza viruses are found around the globe and these viruses easily mutate to new virus variants, the so-called 'antigenic drift'.

In addition, there is the constant threat of a new pandemic influenza virus. A 'new' virus that may be formed after recombination between bird-influenza viruses and pig-influenza viruses, and that is able cause serious disease in humans. A scenario similar to the Spanish flu in 1918 which killed over 50 million people. This is called 'antigenic shift'.

Ideally, an influenza vaccine provides protection against a broad spectrum of seasonal influenza, as well as pandemic influenza viruses. However, current influenza vaccines afford only limited protection against seasonal as well as pandemic influenza. Therefore, new and improved vaccine-strategies are required. This involves new vaccine concepts and improved vaccine production technologies.

At BPRC we use influenza infection models in monkeys to evaluate the protective capacity of novel vaccine strategies.

Research Areas
Influenza

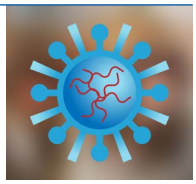
Experimental animal models for universal influenza vaccines

Non-human primate animal models are important to determine whether novel vaccines against influenza can provide a good and broad protection against influenza virus and are safe to use. To measure protection, it is necessary to experimentally expose animals to influenza virus. This is usually done by applying an amount of virus in the nose, mouth and directly in the lungs. However, infection in humans is mainly caused by exposure to aerosols or droplets that enter the airways either via respiration, inhalation, or via contact with contaminated surfaces. To better mimic this typical human way of exposure, a non-human primate model was developed that could be infected by virus in aerosols. Although the animals became infected, the reaction of the body differed from what was observed when the virus was directly injected into the lungs. Infection by aerosols gave lower levels of inflammation and may therefore be more typical of a mild infection in humans. Instead, direct injection in the lungs gave more pronounced inflammatory response, typical of the more severe infection that develops in some humans. These findings can contribute to using the correct animal model for testing of vaccines for use in humans and better prediction of the effect that a vaccine will have in humans. This work is published in Journal of General Virology.

Read more >

Research Areas
Influenza

Experimental animal models for bird flu



The name bird flu, or avian influenza, is misleading as bird flu virus not only infects birds but occasionally also humans. Bird flu virus infection of humans is rare,
but if it happens often fatal i.e 455 deaths in a total of 861 cases for so called H5 viruses.
Like for seasonal flu, it is crucial to have an animal model to test vaccines.

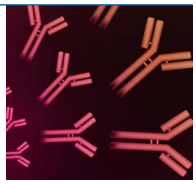
In 2020 we used various techniques to expose macaques to H5N1 bird flu virus.
The results of that work are now prepared for publishing.

Vaccines and antiviral drugs

In 2022 we started a study to investigate a new vaccination regimen against Flu and RSV (Respiratory Syncytial Virus). RSV is the commonest cause of lower respiratory tract infection in children. Worldwide an estimated 34 million children fall ill due to RSV each year and over 3 million children have to be taken to a hospital and 66,000–199,000 children die. We tested whether vaccination via a spray on the tonsils could induce more long-lasting protection against infection with RSV as well as influenza. In addition, we tested whether this vaccine strategy could provide broader protection against influenza, which is needed because of the continuous formation of new virus variants (see above). After an initial intramuscular injection with vaccine, two boosters were administered to the respiratory tract as a spray. Animals were then experimentally infected with RSV. The vaccine strategy proved to result in lower levels of virus replication in the nose, throat, and lungs. In 2023 there will follow an experimental infection with influenza to test whether the vaccine also protects against this virus. When proven effective this new vaccine regimen may also be applied for other respiratory viruses (e.g. SARS-CoV-2).

Research Areas
Influenza

PhD student influenza-specific antibody responses



Protection from influenza infection relies on good antibody responses. As part of a PhD project and in collaboration with the Amsterdam Medical Center novel influenza proteins were designed by a BPRC PhD student, to identify precisely which antibody-producing B-cells are induced by vaccination or influenza virus infection

Read more >

This work describes the method to characterize the diversity of antibody responses against influenza. It will be applied on frozen materials from previously performed animal studies to identify protein specific B cells and antibodies with unknown specificities that could be relevant for vaccine design.

The novel influenza proteins were used to study how B cell responses against influenza are formed during a first infection. This aspect is difficult to study in humans, because the first infection always occurs early in life and adults have already encountered several different viruses. However, it is important to understand how a first response is formed as the first response is thought to determine how one responds to subsequent viral infections.
To study a first response frozen materials from previously performed influenza infection studies in macaques were used.

It is known that after influenza infection most of the antibodies that are formed are directed against the hemagglutinin molecule. Hemagglutinin is present on the outside of the virus particle and is necessary for the virus to bind to its target cells and to infect these cells. Hemagglutinin is composed of a highly variable head domain and a much more constant stem domain. The head domain is needed for the binding to the target cell. It is at this site that most of the mutations occur, leading to new virus variants that are no longer

Research Areas
Influenza

recognized by the antibodies that were formed during a previous infection or vaccination. This is why people need to be vaccinated with a new vaccine every year. The stem domain is much more constant and is needed for virus entry into the target cell. Antibodies directed against this part of the hemagglutinin protein are able to recognize different virus variants, but they are less efficient in blocking the virus than the anti-head antibodies. How to induce these types of antibodies via vaccination is one of the key-questions in influenza vaccine development. We have shown that such antibodies are formed after the first infection with influenza virus. However, we found that they strongly decrease in time, while the anti-head antibodies gradually increase and come to dominate the antibody response. These results were recently published. Further research will be directed at understanding what the effect is of vaccination on these types of anti-stem antibodies.

Read more >

Research Areas

Rabies Virus

Rabies is a devastating viral disease caused by the rabies lyssavirus (RABV). RABV is transmitted to humans via a bite by infected animals, predominantly dogs, but foxes, raccoons, and bat species also serve as natural reservoirs. In humans, once the first clinical symptoms have developed, the disease is uniformly lethal, and patients die in great agony. Rabies causes 58.000 human deaths every year, mostly in rural areas of Africa and Asia. The majority are children of young age (<15 years), who are at high risk from being bitten while playing with animals.

In the last quarter of 2021, we evaluated the immunogenicity of two rabies virus vaccines which were built on the backbones of the attenuated yellow fever virus vaccine strain, YFV-17D, and the Japanese encephalitis virus vaccine strain (CD-JEVAX). The first analyses show that both vaccines effectively induced humoral immunity, equal or exceeding those induced by commercially available rabies vaccines. Further analyses are ongoing, and results will become available in 2022.

In 2022, the analysis from many samples obtained from the animal study performed in 2021 have been finalized and a manuscript is being prepared for publication in 2023.

Research Areas

SARS-CoV-2 and COVID-19

In 2020, the world was startled by SARS-CoV-2, the causative agent of COVID-19. The department of Virology set up a research line to investigate the pathogenesis of this new infection and collaborate in the development of new vaccines to combat the pandemic.

No animal-free alternative

Vaccines stimulate the immune system in such a way that when it encounters the real pathogen, our immune system responds fast and efficient to prevent infection or disease. The immune system is a complicated 'organ' that involves thousands of different cells and molecules. At this point in time, it is not possible to mimic the interplay between all these different components outside a live body. Therefore, efficacy of vaccine candidates is tested in an animal model.

Coronaviruses have limited host range

Coronaviruses, like SARS-CoV-2, are known for their limited host range, but also for their potential to cause serious disease in humans when spilled over from their natural hosts. SARS-COV-2 is not the first coronavirus to cause severe illness in humans. In 2003 there was an outbreak of SARS with a total of 8096 registered infections of which 774 people died. And in 2012 it was MERS with 2494 cases, including 858 fatalities. SARS and MERS are known to infect macaques, and experimental infection results in lung pathology resembling pneumonia in humans. So, when the dramatically fast spread of SARS-CoV-2 became evident, it was clear that a vaccine was urgently needed. With the knowledge that macaques are one of the few animal species susceptible to coronaviruses that cause disease in humans, macaques became one of the preferred animal species to investigate crucial steps in SARS-CoV-2 drug- and vaccine development.

Research Areas

SARS-CoV-2 and COVID-19

SARS-CoV-2 in macaques

In 2020 we established a nonhuman primate model for SARS-CoV-2 infection. One of the first papers showing that macaques were susceptible to SARS-CoV-2 was co-authored by BPRC and published in Science. This study particularly focused on the early events during CoV infection.

Read more >

We have extended this research by studying the post-acute phase of SARS-CoV-2 infection in two macaque species showing ongoing virus replication and pathology in lungs and other tissues even 5 to 6 weeks after infection which could be relevant for understanding the long-term consequences (like Long-COVID syndrome) of COVID-19 in humans.

Read more >

As an extension of this study, we investigated the neuropathological changes that occurred after a SARS-CoV-2 infection in the brains of macaques. Our data highlight the potential of the virus to cause pathology in the brain of macaques as in all animals we observed a range of neurological abnormalities, like hypermetabolic pituitary gland, α-synuclein inclusions, activated microglia in the brain parenchyma, and infiltrating T-cells. The heterogeneity of these manifestations in the brains shows the neuropathological potential of SARS-CoV-2 and should be considered a warning for long-term health risks, following SARS-CoV-2 infection.

Read more >

Research Areas

SARS-CoV-2 and COVID-19

Vaccines and antiviral drugs

In 2021, we tested several SARS-CoV-2 vaccine candidates in collaboration with both academia and pharmaceutical partners. One of the first-generation vaccine candidates that was evaluated at BPRC has been approved by different national and international authorities for use in humans. This vaccine is now used worldwide as one of the first effective SARS-CoV-2 vaccines developed. The results of this efficacy study have been published in the Journal of Experimental Medicine.

Read more >

Four other SARS-CoV-2 vaccine candidates (2nd generation) have been evaluated in 2021 as well. Some vaccines were very effective in protecting against SARS-CoV-2 infection in our models. Several studies will be published in 2022.

In 2022, we performed 2 studies using different new vaccines candidates under the sponsorship of the European TransVac-2. One study was done in collaboration with a consortium led by the University of Copenhagen (Denmark). The second study was in collaboration with a small biotech company (Belgium). Both efficacy studies were done in 2022, but data analyses are still ongoing. We intend to publish the data in 2023.

Research Areas
HIV-AIDS

AIDS is caused by an infection of the Human Immunodeficiency Virus (HIV). Antiviral medications can delay HIV growth and can prevent AIDS development. Furthermore, these compounds can prevent transmission from the virus from an infected person to a non-infected individual. However, we see that those antivirals are not able to prevent the spread of this pandemic worldwide as there are approximately 1.5 million newly infected people each year. Therefore, we hope that effective vaccines against HIV are more efficient in stopping the spread of HIV. Already more that 35 years have been passed to find an effective vaccine. Unfortunately, no effective vaccines have been developed yet, but the search continuous.

The HIV prime-boost vaccination study against HIV, which started in 2020 as part of a large EU consortium, has been finalized this year. Despite the use of newly developed vaccine candidates (and the vaccination strategy tested), they failed to induce strong immune responses in the rhesus macaques. The candidates tested did not induce stronger and/or broader immune responses as compared to earlier evaluated vaccine candidates.
Still in vitro analysis is ongoing to investigate the (lack of) effectiveness of these latest vaccine candidates against HIV.

Research Areas

Mosquito-borne Diseases

Dengue virus, West Nile virus, Rift Valley fever virus, and Zika virus are mosquito-borne viruses that cause an infection in people. In most people, the infection is transient and without clinically relevant illness. However, approximately 1% of the patients suffer from complications. Nowadays, over 700 million people get infected with a mosquito-borne virus each year. Due to the growth of the human population and global warming this number is expected to increase dramatically over the next decades.

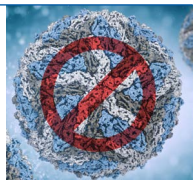
So far, vaccines are only available for dengue virus and yellow fever virus, but these vaccines have severe limitations and are not advised to people that run the highest risk for complications, namely children, older people and those people with an impaired immune system.

BPRC has developed several infection models to investigate mosquito-borne viruses. In 2020 these models were mainly used for proof of concept-studies for vaccines and antiviral drugs.

Research Areas

Mosquito-borne Diseases

An antiviral drug for dengue virus



There are four types of dengue viruses. Each of them inducing a somewhat different set of antibodies after infection. A first infection results in lifelong protection against this specific serotype. However, if the second infection occurs with another serotype the patient has an increased chance to develop severe disease. This phenomenon is called antibody-dependent enhancement (ADE). Severe dengue disease is a potentially deadly complication due to plasma leakage, fluid accumulation, respiratory distress, severe bleedings, or organ impairment. Every year, half a million patients require hospitalization and about 20,000 people die of severe dengue.

With a European partner we evaluated in 2021 their antiviral compound against dengue virus type 3 infection in our animal model. This compound had been tested in 2020 against one of the four dengue virus serotypes and proved very effective against serotype 2. This year we evaluated the same compound but now against serotype 3 dengue virus infection to determine its broad effectiveness.

In 2022, we continued together with our partner the evaluation of their antiviral compound, now against dengue virus type 4 to determine its broad effectiveness. The most important results from this collaboration have been published early 2023.

Read more >

Research Areas

Mosquito-borne Diseases

A new zika virus vaccine tested in rhesus macaques

The classic method of vaccine production is time consuming, requires batch-to-batch evaluation and distribution using a cold-chain. This hampers a rapid response in case of an outbreak. The 2015-2016 outbreak of Zika virus brought the weaknesses of classic vaccines to light.

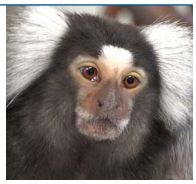
A new generation of vaccines may overcome these problems. DNA vaccines are safe, can be rapidly produced and do not require a cold-chain. However, not all DNA vaccines are effective in inducing an immune response.

BPRC has collaborated in an EU-funded project to perform a proof-of-concept study in monkeys with a DNA vaccine encoding for the Zika envelope protein. Although the immune responses were high, the vaccine did not induce sterile immunity. A manuscript describing this study is currently in preparation.

Research Areas

Mosquito-borne Diseases

A new Rift Valley fever virus vaccine tested in common marmosets



Rift Valley fever virus (RVFV) is an emerging mosquito-borne virus that is highly pathogenic to wild and domesticated cattle and humans. While animals are exclusively infected via mosquito bites, humans can also be infected via contact with tissues or blood released during the slaughtering of RVFV-infected animals. No human vaccine is available and currently commercialized veterinary vaccines are not optimal for human use.
In collaboration with a Dutch partner, we tested new vaccine candidates for safety and their capacity to generate protective immune responses in common marmosets.
The marmoset was used because it is very susceptible to the virus, so that any safety risks can be adequately measured. In addition, testing in a non-human primate species makes it possible to better extrapolate the measured immune responses to the human situation.
The vaccines were found to be safe and to induce strong potentially protective responses.

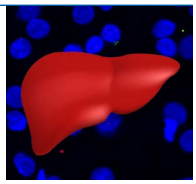
The analysis from data from this study was finalized in 2022, resulting in the publication of this project.

Read more >

Research Areas
Malaria

There are 5 malaria parasite (Plasmodium) species that infect humans, of which *P. falciparum* and *P. vivax* are the most important. *P. vivax* uniquely forms dormant parasite stages in the liver, called hypnozoites. Hypnozoites are only formed in 5 primate malarias (including 2 human malarias) and we use *P. cynomolgi*, that infects rhesus monkeys, as a model for *P. vivax*. We study hypnozoite biology, interactions with the host to develop new diagnostic methods for hypnozoite infections and we work on discovery of new drugs, that are urgently needed to kill hypnozoites. For these studies we have developed an *in vitro* liver stage culture system for *P. cynomolgi*, as well as technology to genetically modify the parasite.

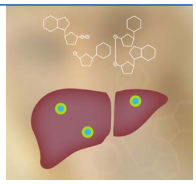
Progress in drug discovery for hypnozoites



We routinely use in vitro cultures of liver cells that are infected with *P. cynomolgi* malaria parasites to test whether liver stage parasites (and in particular the dormant subset of parasites) are sensitive to potential new antimalarial compounds.
The analysis of these experiments was until shortly performed by microscopy, distinguishing the antibody-stained growing and dormant parasites by shape/size. We have developed a new method for the analysis using transgenic parasites that express bioluminescent markers, which can be used for parasite detection. By treating the infected liver cells with a compound that kills only the growing liver stage parasites, we can specifically detect the dormant stages. This dormant liver parasite population can be used for screening with compounds to detect anti-hypnozoite activity. We have thus far tested 8,000 compounds with this method, including the 400 compound 'pathogen box' (provided by MMV, medicines for malaria venture). In this pathogen box we identified one compound that was marked as a hit in several tests (both microscopy-based and bioluminescence-based), this compound will be investigated more thoroughly.

Research Areas
Malaria

Towards the rapid diagnosis of *Plasmodium vivax* malaria hypnozoite infection



In 2022, the second phase of the series of two in vivo experiments, which started in 2021 as part of a metabolomics study funded by the GHIT Fund (Global Health Innovative Technology Fund), was carried out. This was part of a larger project which is aimed at developing a rapid test for identifying people that are “latently” infected with malaria and also included a preliminary *in vitro* phase to test feasibility.
At the end of the first *in vivo* study, an independent go-no go decision was carried out by the GHIT Fund to determined whether the results obtained in the first *in vivo* study were promising enough to allow the team to proceed with the second *in vivo* study.

The first *in vivo* study was aimed at identifying unique metabolic signatures in the serum of animals “latently” carrying dormant malaria infection stages (hypnozoites). Approximately 40 metabolites in the serum that were selectively expressed in the dormant stage (hypnozoite) condition only were identified. The second phase was aimed at confirming such metabolic signatures and determining the sensitivity of the metabolite approach *in vivo*. 27 of the 40 metabolites identified in the first *in vivo* experiment were successfully confirmed in the second phase and 19 metabolites were selected for further analysis as they are the most promising in terms of developing a diagnostic kit for *Plasmodium vivax* malaria hypnozoite infection in humans to be deployed under the field conditions found in endemic countries.

Research Areas

Neurobiology & Aging

In 2022 the department Neurobiology & Aging was joined by two technicians and a postdoc, whereas to former members left the BPRC for jobs elsewhere. The department further developed it's work on aging and dementia, and further explored the effects of SARS-CoV-2 infections on the brain.

Background

Aging

As people age, they become more susceptible to various health conditions, including neurodegenerative diseases. Neurodegenerative diseases are a group of disorders that progressively affect the function and structure of the brain and nervous system, leading to cognitive decline and functional impairment. These diseases are often chronic, disabling, and incurable, and can significantly impact the quality of life of affected individuals and their families.

There has been a significant increase in the incidence of neurodegenerative diseases in recent years, particularly in developed countries. This increase can be attributed to several factors, including the aging of the population, changes in lifestyle, and improved diagnostic capabilities. As life expectancy continues to rise, the prevalence of neurodegenerative diseases is expected to increase further, putting a significant strain on healthcare systems and society as a whole.

Research Areas

Neurobiology & Aging

Neuroinflammation

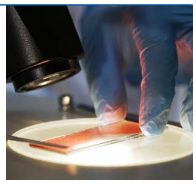
Neuroinflammation is a type of inflammation that occurs in the brain and spinal cord in response to injury, infection, or disease. Normally, inflammation is a necessary response of the immune system to protect the body from harmful stimuli, such as pathogens or toxins. However, when inflammation becomes chronic or excessive, it can lead to tissue damage and contribute to the development or progression of various diseases, including neurological disorders.

During neuroinflammation, immune cells in the brain, microglia and astrocytes, become activated and release pro-inflammatory molecules, such as cytokines and chemokines. These molecules trigger a cascade of events that can further recruit peripheral immune cells to the site of injury or damage. Additionally, neuroinflammation is associated with a wide range of neurological disorders, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, and traumatic brain injury. In these conditions, neuroinflammation is thought to play a role in the degeneration of neurons and progression of disease. Additionally, aging, chronic stress, obesity, and other lifestyle factors have also been linked to increased neuroinflammation, which may contribute to cognitive decline and other symptoms. Inflammation in the brain can be regarded as a double-edged sword; it is necessary for repairing damaged tissue and fighting infections while on the other hand controlling inflammation is critical for a delicate balance between an appropriate immune response and excessive inflammation leading to collateral damage.

Although we know aging is a key contributor to the progression and aggravation of neurodegenerative diseases, it is challenging to determine their exact relationship. By analysing brain samples of different species and different ages, and their blood factors, including proteins and immune cells, we aim to get a deeper understanding of how aging may contribute to neurodegenerative diseases, but also neurological disorders due to viral infections, such as SARS-CoV-2.

Research Areas

Neurobiology & Aging



Age-related blood factors

Aging comes with a lot of changes throughout the body, of which many leave an imprint in the blood composition. As previous research has shown that changes in the blood composition can have an effect on various organs, including the brain, we aimed to identify how the blood composition changes with age in humans.

To do so, we integrated the results of four human plasma proteomic datasets. By combining this information, we were able to compare age-associated changes of ~5000 proteins in the plasma of individuals between the age of 16 and 100. Across these datasets, we identified a set of Aging Proteins (APs) which showed similar age-associated effects and validated these effects in other independent studies and methods. APs were found to be highly associated with various age-related diseases, such as organ failure and dementia. Moreover, the expression levels of these APs in the plasma were found to be good predictors of chronological age, even across independent datasets. However, some individuals showed a discrepancy between their chronological age and estimated biological age based on their plasma proteome. To further investigate this difference we identified several candidate proteins that may contribute to the acceleration of the aging process, or may actually slow it down. These results will be published in 2023.

Currently, we are translating and validating our findings in the Rhesus Macaque (*Macaca Mulatta*). We performed proteomics on plasma samples from our own macaque colony spanning a broad age range, and identified similarities in several human APs and the aging Macaque plasma proteome. To gain a better understanding of brain aging, we selected APs most likely reflective of this process as follow-up candidates to compare transcriptomic and proteomic changes in brain tissue. Using this approach we aim to gain a better mechanistic understanding of brain aging, which may ultimately lead to novel therapies to remain healthy and cognitively fit for as long as possible.

Research Areas

Neurobiology & Aging

Aging brain collection

To study various aspects of brain aging, we have started to collect, dissect, and store the brains of non-human primates in a structural manner. With this method we are currently setting up a biobank of invaluable brain material for research on the aging brain and neurodegenerative disease. This material can be used for research interests of the BPRC but can also be utilised by external parties for research collaborations.

Over the last year we have dissected 75 brains and stored over 2700 different blocks, both snap frozen as well as formalin fixed paraffin embedded (FFPE). The brain biobank is realised from our current efforts to collect as many brains as possible from the NHPs in the BPRC. In this way we are generating a collection that contains the brains of normal aging NHPs but also brains of NHPs during disease.

Research topics using the aging brain collection:

Neuroinflammation

One of the research areas that is currently being focused on is the heterogeneity of microglia during aging. Microglia are the resident immune cells of the central nervous system and play a critical role in the maintenance of brain homeostasis and are the main driving force of inflammatory processes. Over the years, research has revealed that microglia are not a uniform population of cells, but rather a heterogeneous group with diverse functions and properties. Microglia can vary in morphology, gene expression, and response to stimuli depending on their location within the brain, developmental stage, and disease state. Understanding the heterogeneity of microglia is important for developing targeted therapies for neurological disorders and improving our understanding of the complex interactions between microglia and other cells in the brain. For this we are analysing the morphology and states of microglia throughout the lifespan in NHPs by

Research Areas
Neurobiology & Aging

utilising the blocks generated in our brain biobank. We have set up a pipeline for microglial morphology analysis to analyse many different parameters regarding the complexity and the shape of microglial cells.

Neuropathology

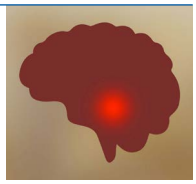
Due to the increase in the incidence of neurodegenerative disease we are investigating whether aging NHPs develop similar pathology in the central nervous system that is also observed in humans. Neurodegenerative diseases are characterized by pathology in the brain, for example Alzheimer's disease is accompanied by the formation of Aβ plaques and tau tangles in the cortex. As these diseases progress, the pathology spreads throughout the central nervous system leading to neurological complications and loss of quality of life. To establish whether NHPs can be used for research on neurodegenerative diseases we have started to investigate if pathology associated with ageing and neurodegenerative diseases can be found in ageing macaques.

Overall, we aim to determine how similiar aging processes are between non-human primates and humans.

Research Areas

Neurobiology & Aging

SARS-CoV-2 induced neuroinflammation and neurological consequences



Coronavirus disease 2019 (COVID-19) patients initially develop respiratory symptoms, but they may also suffer from neurological symptoms. People with long-lasting effects after acute infections with severe respiratory syndrome coronavirus 2 (SARS-CoV-2), i.e., post-COVID syndrome or long COVID, may experience a variety of neurological manifestations. Although we do not fully understand how SARS-CoV-2 affects the brain, neuroinflammation likely plays a role.

In 2022 we published a paper on our first SARS-CoV-2 infection study in which we describe neuroinflammatory processes in the brains of four cynomolgus and four rhesus macaques infected with the Alpha variant of SARS-CoV-2.

Read more >

We continued to investigate the effects of SARS-CoV-2 infection on the brain in four rhesus macaques that were infected with the Delta variant. Because neuroinflammation was apparent in the first SARS-CoV-2 study we imaged these four macaques longitudinally with a PET-CT tracer more specific to neuroinflammation, namely [^{18}F]DPA-714, which targets the translocator protein TSPO. This study revealed an increased TSPO tracer uptake throughout the brain of all infected animals already from 2 days post infection until approximately 30 days post infection, suggesting neuroinflammation is an active and continuous process even weeks after the acute infection period. Postmortem immunohistochemical analysis showed increased TSPO expression and a clear response of the glial cells in various brain regions of SARS-CoV-2 infected animals. These results will be published in 2023. We are currently further exploring SARS-CoV-2 tropism, the mechanism behind these neuroinflammatory processes, and the neurodegenerative consequences.

Outreach

Biomedical research is not a goal. Our goal is to understand diseases and find a cure. We cannot do that alone. That is why we share our results and discuss them with other scientists. Together we know more and that brings us closer to the solution.

We (co-)authored

50 scientific publications in peer reviewed journals.

Find the complete list here.

we gave lectures

to train students

at Dutch universities and HBOs on possibilities and restrictions of working with monkeys in research.

We actively

feed our website

with relevant updates.

We organized

bachelor, master & PhD courses

for future scientists.

During (online)

(inter)national meetings

we present and discuss our results.

We also organize

popular scientific lectures

in understandable Dutch for all BPRC staff.

We active participate in

meetings discussing alternatives

for animal experimentation.

We supervise and train

PhD, master & bachelor students.

After graduation they apply their knowledge elsewhere and contribute to science.

As partner in various research networks

our work is publicly available

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Biomedical Primate
Research Centre