BASR
2021

Biomedical Primate Research Centre
Annual Scientific Report 2021

Welcome
Join our journey through health research and alternatives

You're looking at a microscopic image of ramified microglia in cell culture. Photo: R. Timmerman

Biomedical Primate Research Centre

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Monkey Research

- Explained -

Welcome to the 2021 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, 2021, BPRC housed 1055 monkeys, 677 rhesus macaques (*Macaca mulatta*), 234 cynomolgus monkeys (long-tailed macaques; *Macaca fascicularis*) and 144 common marmosets (*Callithrix jacchus*). On December 31st, 2021, BPRC housed 985 animals, 638 rhesus macaques, 240 long-tailed macaques and 107 common marmosets. In 2021 BPRC worked with 211 animals, 196 rhesus macaques, 13 long-tailed macaques and 2 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?

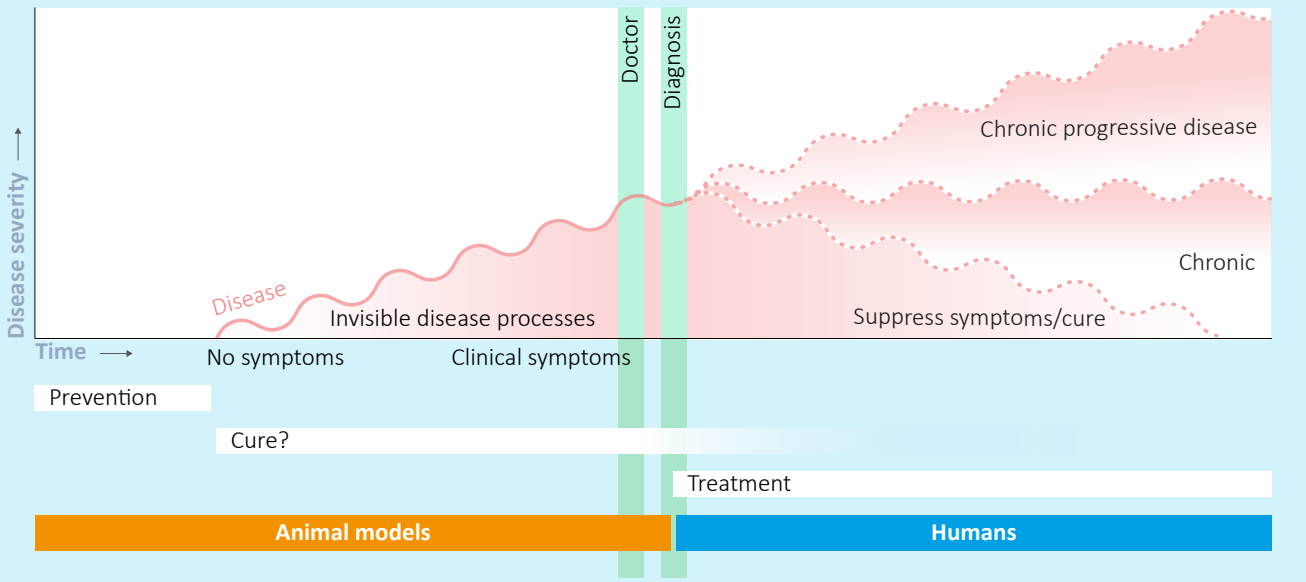
BPRC focuses on life threatening and/or debilitating diseases that affect millions of people. Diseases without cure or treatment because the complicated disease mechanisms are not yet fully understood. The European Commission concluded that the type of research conducted at BPRC cannot be done without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



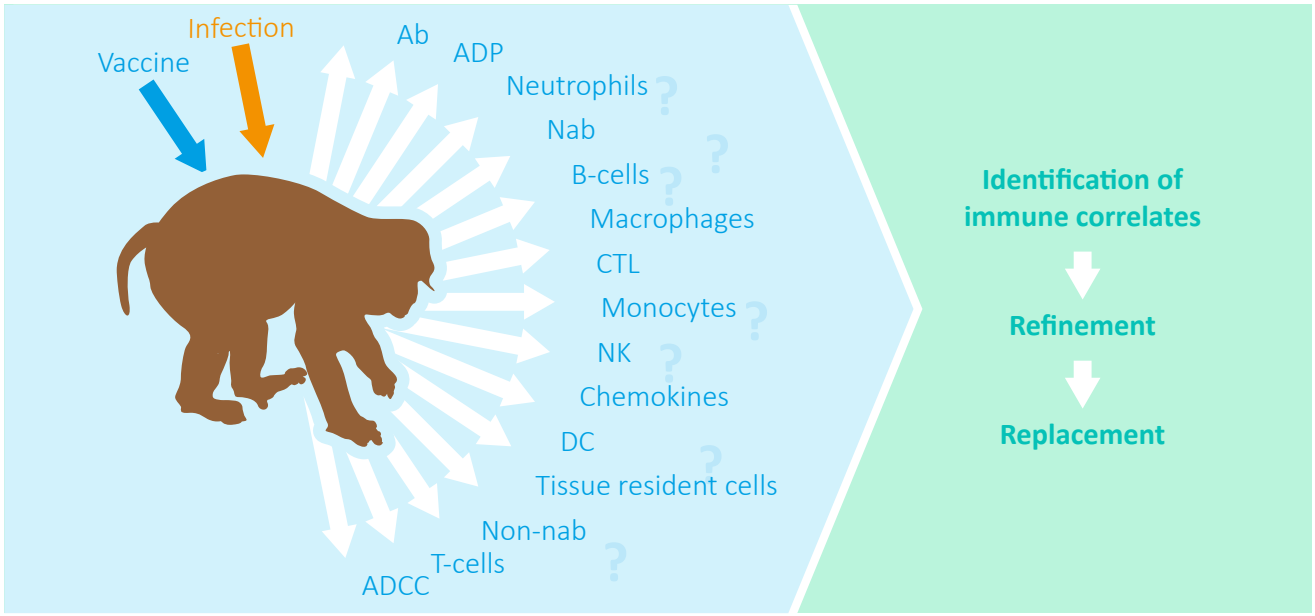
The X-axis represents the time and y-axis disease severity. A disease does typically not start with clinical symptoms. The onset is often without signs. But as time progresses the damage to cells and organs accumulate and cause clinically relevant disease. Depending on the disease this can take days 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:

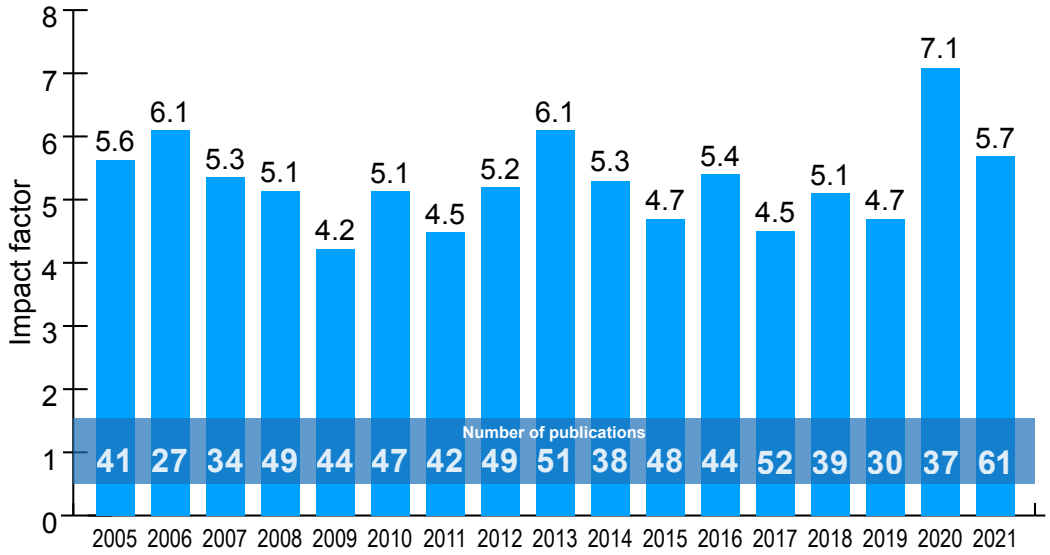
More information regarding animals in experiments is found at Stichting Informatie Dierproeven, European regulatory bodies (Directive 2010/63/EU), the Dutch law (Wet op de dierproeven). The Centrale Commissie Dierproeven (CCD) is the legal body in The Netherlands that is authorized to provide licenses. 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 2021 was again mainly shaped by the corona pandemic and its consequences. We did a considerable amount of research on various COVID-19 vaccine candidates, therapeutics and developed models for various variants of concern. We are very proud that BPRC participated as a partner in delivering one of the first globally used corona-vaccines to the community.

In July, the dedicated work of our staff and the importance of preclinical research in monkeys in the context of pandemic preparedness was show-cased by the Volkskrant, one of the mainstream Dutch newspapers. We got a lot of positive feedback from society for our transparent attitude and the dedication of our staff members.

We did, however, conduct considerable amounts of work on other infectious diseases and published for instance important papers on Tuberculosis, malaria and Flu. Also in the field of animal free research and refinement of animal models some hall mark papers were published.



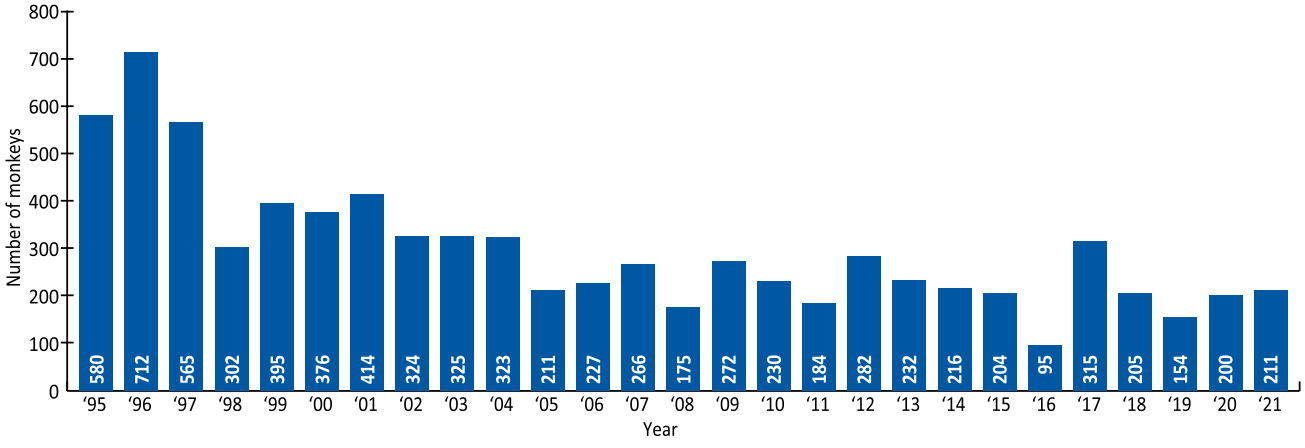
Message from the board

Despite the high work pressure we invested in animal welfare projects and in new staff to facilitate, for instance, investigation of the impact of coronavirus infection on the central nervous system which may be a partial explanation for the long Covid syndrome.

Altogether we published 61 manuscripts in the peer reviewed scientific literature with an average impact factor of 5.75.

One of the other highlights of 2021 was that one of our staff members did defend his PhD thesis at the Utrecht University and passed the exam cum laude.

This year was due to all the hard work financially sound and we closed the books with a positive result of 4000 euro. Most of our main accomplishments have been published on our website and are listed under the subject news items.



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

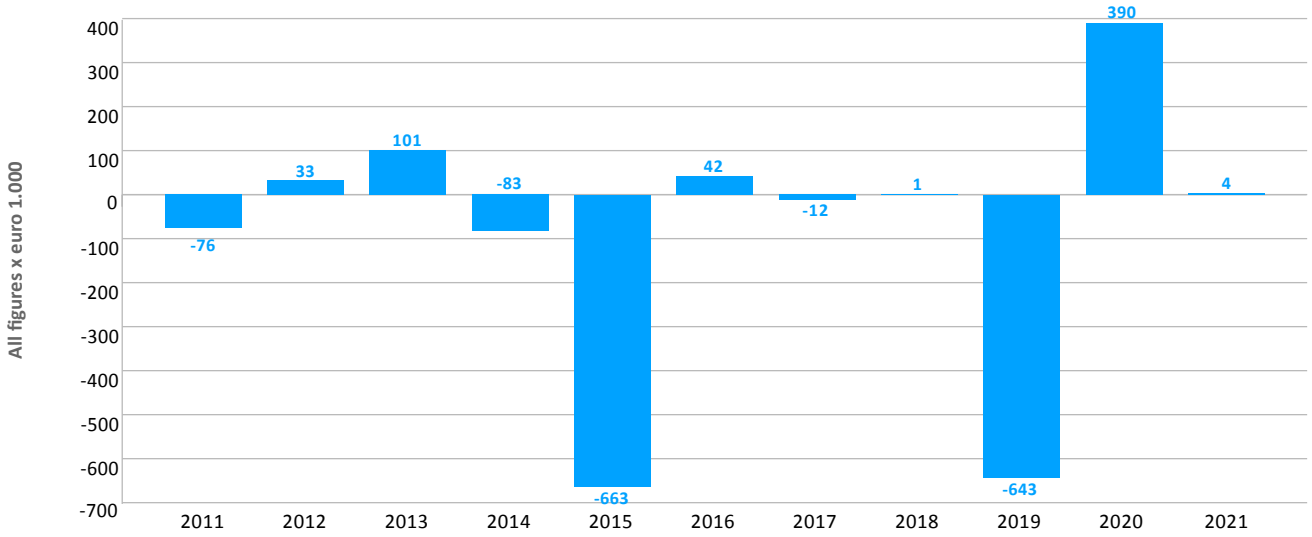
Our Financial Results

Foundation Biomedical Primate Research Centre (BPRC) closed the fiscal year 2021 with a positive result of 4 K€.

Total turnover projects increased from 13,1 million euros in 2020 to 13,9 million euros in 2021, a difference of 0,8 million euros. The increase in income commissioned by third parties is caused by the COVID19 pandemic and the emergence of different virus variants, which has led to many new research assignments for the BPRC.

The operational costs are eight percent higher than the budget for the year 2021, as a result of higher price increases for material purchases for conducting research and higher level of maintenance costs.

Result development BPRC 2011-2021



Our Financial Results

	2021 (K€)	2020 (K€)
Turnover projects (extern)	5.174	4.812
Turnover projects (subsidy)	8.375	8.059
Total turnover projects	13.549	12.871
Other excluding interest	353	266
	353	266
Total turnover	13.902	13.137
External direct project costs	578	830
Staff costs	8.276	7.748
Depreciation	742	648
Other operating charges	4.237	3.501
Total operating costs	13.833	12.727
Profit/loss on ordinary activities	69	410
Interest	65-	19-
Profit for the financial year	4	391
Tax	-	-
Profit for the financial year after tax	4	391

EFFECTIVE PERSONNEL

	2021		2020	
Service Departments	15,8	15%	16,3	16%
Animal Sience Department	44,2	43%	41,3	42%
Research	43,2	42%	41,8	42%
Total	103,2	100%	99,4	100%

	2021 (K€)		2020 (K€)	
ASSETS				
FIXED ASSETS				
Buildings and structures	25.342		27.249	
Tangible fixed assets	1.603		1.780	
		26.945		29.029
CURRENT ASSETS				
STOCKS		72		87
DEBTORS DUE WITHIN ONE YEAR				
Work in progress	1.752		1.025	
Receivables from contracts	973		540	
Receivables tax	17		18	
Other receivables	235		97	
		2.977		1.680
Cash at bank and in hand		13.294		15.229
Total assets		43.288		46.025

	2021 (K€)		2020 (K€)	
LIABILITIES				
EQUITY				
Equity	4.508		4.117	
Revaluation reserve buildings	5.388		5.906	
Result current year	4		391	
		9.900		10.414
PROVISIONS				
Primates	-		-	
Deferred tax liabilities	-		-	
(Flexibel) retirement	-		-	
Repairs buidings	1.910		1.872	
		1.910		1.872
LONG TERM DEBTS				
Bank	19.099		20.236	
Received in advance on asstes	6.034		6.512	
		25.133		26.748
SHORT TERM DEBTS				
Received in advance on projects	1.609		2.500	
Received in advance on assets	369		323	
Received in advance subsidy	700		908	
Accounts Payable (TAX)	456		414	
(Flexibel) retirement	103		-	
Accounts Payable	789		723	
Commitment Bank	1.138		1.083	
Other liabilities	1.181		1.040	
		6.345		6.991
Total liabilities		43.288		46.025

Our Financial Results

WNT-Verantwoording Organisatie BPRC

BEZOLDIGING TOPFUNCTIONARISSEN

Leidinggevende topfunctionarissen

Gegevens 2021	
Bedragen X 1€	
Functie(s)	Directeur
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
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT
Bezoldiging	193.313
Reden waarom de overschrijding al dan niet is toegestaan	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT

Gegevens 2020	
Aanvang en einde functievervulling in 2020	1/1 - 31/12
Omvang dienstverband (in fte)	1,0
Dienstbetrekking	Ja
Bezoldiging	
Beloning plus belastbare onkostenvergoedingen	157.564
Beloningen betaalbaar op termijn	36.215
Subtotaal	193.779
Individueel toepasselijk bezoldigingsmaximum	201.000
Bezoldiging	193.779

Toezichthoudende topfucntionarissen

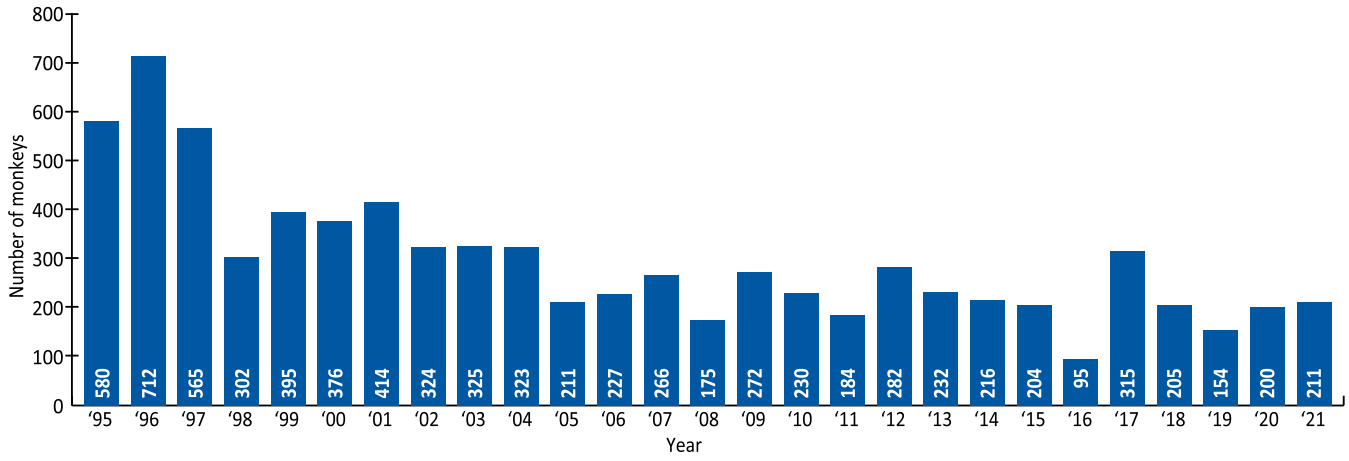
Gegevens 2021					
Bedragen X 1€					
Functie(s)	Lid Raad van Toezicht (Voorzitter)	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht
Aanvang en einde functievervulling in 2021	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12		1/1 - 31/12
Bezoldiging					
Bezoldiging	9.278	6.959	6.655		6.959
Individueel toepasselijk bezoldigingsmaximum	31.350	20.900	20.900		20.900
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 2020				
Aanvang en einde functievervulling in 2020	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging	9.278	6.959	6.655	5.066
Individueel toepasselijk bezoldigingsmaximum	30.150	20.100	20.100	20.100

Gegevens 2021				
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 2021		1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging				
Bezoldiging		6.655	6.959	6.959
Individueel toepasselijk bezoldigingsmaximum		20.900	20.900	20.900
Onverschuldigd betaald en nog niet terugontvangen bedrag		NVT	NVT	NVT
Reden waarom de overschrijding al dan niet is toegestaan		NVT	NVT	NVT
Toelichting op de vordering wegens onverschuldigde betaling		NVT	NVT	NVT

Gegevens 2020			
Aanvang en einde functievervulling in 2020	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging	6.655	6.655	6.959
Individueel toepasselijk bezoldigingsmaximum	20.100	20.100	20.100

Facts & figures



The number of monkeys we worked with over the years and the scientific achievements in 2021.

A total of

61 scientific publications

in peer reviewed international journals.
Complete list

One

PhD student defended his thesis

with honors (cum laude).

In addition we

train seven PhD students

to write and defend their thesis.

We placed

37 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 2021.

Chemical stock(s)

In 2021 we organized an awareness program to reduce the amount of (older) chemicals on-site and make more use of shared stocks between labs/facilities to minimize the need for multiple stocks of the same compound.

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 an increased metabolic activity. Increased metabolic activity can be due to cancer, infection/

Research Areas
Alternatives

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.

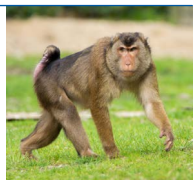
Alternative methods are categorized along the principle of the 3Rs of Replacement Reduction and Refinement, all of which have a place within BPRC. BPRC is committed to the implementation of the 3Rs and is actively involved in the development and implementation of 3Rs methods. The 3Rs are implemented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

Distributed and chopped food improve welfare of mangabeys

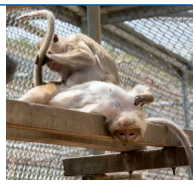


Not only diet, but also the distribution and size of food can affect animal welfare. Indeed, the mangabeys of Rotterdam Zoo reacted better to distributed and chopped food.

The white-naped mangabey is an endangered and rare zoo species, yet little is known concerning their welfare in captivity. The assessment of welfare should incorporate a net balance of negative and positive welfare behavioural indicators. These behaviours, and thus welfare, can be affected by the way food is presented based on its distribution, clumped or dispersed, and its size, chopped or whole. This study investigated the effect of food presentation on time-budget behaviours and stress-related measures, in four crossed-over test conditions of food distribution. The group-living mangabeys of Rotterdam Zoo were provided with vegetables that differed in distribution and size: clumped-chopped, dispersed-whole, dispersed-chopped, and clumped-whole. Mangabeys spent least time being inactive and subordinates and juveniles spent most time foraging during the dispersed-chopped condition, while the reversed was found during the clumped-whole condition. In addition, mangabeys stole food more often and engaged in less self-directed behaviours during dispersed-chopped, compared with dispersed-whole. In contrast, food distribution and size did not affect aggression, play, activity, self-grooming and diarrhea. Consistent with most of the literature, chopped, dispersed items appeared to be the best, whereas presenting whole food items appeared to be the worst for welfare.

Read more >

Research Areas
Ethology



How to gain and maintain friendships

Friendships in primates and humans result from repeated friendly interactions. This study explores, by employing a computer model, how these friendly events update the long-term feelings of friendship. Unexpectedly, when starting a friendship requires many friendly interactions, the friendships that do develop are more stable.

Emotional bookkeeping is the process by which primates integrate the emotional effects of social interactions to form internal representations of their affiliative relationships. The dynamics and speed of this process, which comprises the formation, maintenance and fading out of affiliative relationships, are not clear. Empirical data suggest that affiliative relationships are slowly formed and do not easily fade out. The EMO-model, an agent-based model designed to simulate the social life of primates capable of emotional bookkeeping, was used to explore the effects of different types of internal relationship dynamics and speeds of increase and decrease of relationship strength. In the original EMO-model the internal dynamics involves a fast built-up of a relationship independent of its current quality, alongside a relatively fast fading out of relationship quality. Here we explore the effect of this original dynamics and an alternative dynamics more in line with empirical data, in combination with different speeds of internal relationship quality increase and decrease, on the differentiation and stability of affiliative relationships. The alternative dynamics leads to more differentiated and stable affiliative relationships than the original dynamics, especially when the speed with which internal relationship quality increases is low and the speed with which it decreases is intermediate. Consequently, individuals can groom different group members with varying frequency and support a rich social life with stable preferred partners and attention to several others. In conclusion, differentiated and stable affiliative relationships are especially formed when friends are not made too quickly and not forgotten too easily.

Read more >

Research Areas
Ethology

Human social organisation has all features that promote social learning

Social learning from others can safe energy and reduce risk. Social learning is also the basis of culture. In this review, we explored what features of social organisation promote social learning.

Social learning, which is a mechanism that allows an individual to acquire skills from other individuals, occurs in a social context. Therefore, factors that influence social context, like social structure, will impact social learning opportunities. This review explores how features of social structure affect social learning opportunities in primates, either through their relationship with social tolerance or through the number of social learning models. Features that are investigated in this review and that we hypothesize affect social learning opportunities are parental investment, dominance hierarchy, nepotism, social bonds, dispersal, group size, fission-fusion dynamics, and sex ratio. For most of these features we find evidence, but support varies. Of all primate species, only humans show all the requirements of an optimal social structure to promote social learning. Future research into social learning and culture should not overlook the social context in which it takes place.

Read more >

Photo credit: Animal Behaviour & Cognition, Utrecht University

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.

Animal health

The animals in the BPRC breeding colony have free access to indoor and outdoor facilities. In general, indoor Air Quality (IAQ) is strongly associated with animal health and wellbeing. To check if the indoor environment of macaques (*Macaca spp.*) at the BPRC is optimal and does not have potential negative effects on the health of both animals and staff, we assessed the IAQ. This research was done in collaboration with expert groups on air quality from the Veterinary Faculty of Utrecht University. The temperature, relative humidity and concentrations of inhalable dust, endotoxins, ammonia and fungal aerosols were measured at stationary fixed locations in indoor enclosures of group-housed rhesus (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*). In addition, the personal exposure of caretakers to inhalable dust and endotoxins was measured and evaluated. Data are currently evaluated to assess if and where adaptations are needed and to prepare publication.

In all non-human primate colonies, individuals can be found that suffer from chronic or intermittent diarrhea, which is difficult to treat. A long-term study has been started to investigate if different diets can influence the occurrence of diarrhea. Several non-human primate specific diets are currently tested. The data will become available during 2022. To further improve the health of the animals, group size in our breeding colonies was decreased. Over the last few years, average group size changed from 15 to 11 individuals in longtailed macaque groups and from 21 to 16 individuals in rhesus macaque groups.

Research Areas

General Primate Biology & Welfare

In 13 out of 26 rhesus breeding groups a breeding male was present, resulting in 102 births. In the longtailed macaque breeding groups 17 babies were born. The BPRC marmoset breeding colony had 5 breeding couples. To reduce the number of births, but leave the breeding families intact, some females were implanted with Implanon just before or within the first week after parturition. Implanon has no effect on delivery or lactation, and resulted in only 6 births.

Observations and training of animals

To ensure animal well-being, behavioural observations and training of animals are very important topics at the BPRC. Recently, we have installed cameras, 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. When unrelated individuals are introduced to each other, information can be obtained without disturbance by humans. The use of these camera's also gives insight in the sleeping pattern and night activity of the animals. This makes assessment of the compatibility of a pair much easier and more reliable.

Due to COVID precautions, positive reinforcement training (PRT) of animals in the breeding colonies was minimized, and for a large part of the year completely paused. The importance of this training became apparent; animals behaved less anxious and were less stressed after PRT training was resumed.

In the experimental facility, we train animals to cooperate with various specific procedures. This year the animal trainers succeeded in training one rhesus macaque to cooperate in a voluntary blood withdrawal protocol. When it was certain the animal was fully cooperating with the procedure, and presented his leg without hesitation, a Vascular Access Port (VAP)

Research Areas
General Primate Biology & Welfare

was surgically implanted in the lower leg of the animal. Blood withdrawals were taken several times. Unfortunately, due to some technical difficulties the device didn't work as planned. The technique however is very promising, and more animals will be trained and implanted with a VAP in 2022.

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. Several studies explore how husbandry, overweight and housing are related. Bodyweight is an important health and welfare indicator for captive non-human primates (NHPs). Being able to weigh animals regularly without having to handle or train them is thus desirable for monitoring animal health and welfare. We investigated the reliability of voluntary weighing in large social groups of rhesus macaques.

Read more >

A scale was placed in their home enclosure during several sessions and 68% of the individuals stepped onto the scale. The level of agreement between bodyweight by voluntary weighing and bodyweight measured during sedation was very high. Thus, voluntary weighing can be used to monitor body weight. A further study explored the effect of diet on bodyweight and health parameters. In the long-tailed macaque breeding colony, provisioning of bread was replaced by grains and vegetables, while the basic diet of monkey chow remained the same.

Read more >

Research Areas
General Primate Biology & Welfare

Overweight status did not differ after dietary change, but some biochemical parameters related to glycemic response and lipid metabolism improved. Next, in 32 female rhesus macaques the effect of a change from group- to pair-housing on cortisol, as measure for stress, and body fat levels was determined.

Read more >

Besides individual differences, cortisol levels were higher in pair-housing compared to group-housing. Body fat levels did not differ between housing conditions. Accordingly, there was no clear association between cortisol and body fat levels. These studies clarify the link between husbandry, overweight and housing.

Behaviour

New males can best gradually enter a multigenerational group of macaques: a manual

Mimicking behavioural patterns of wild macaques improves welfare, yet can also have it challenges. Preventing inbreeding when housing macaques in stable multigenerational groups requires the introduction of unrelated males. The paper provides the crucial steps for an often-successful introduction method and recommends a gradual introduction of the new male into the groups of resident females.

Housing of primates in groups increases animal welfare; however, this requires management to prevent inbreeding. To this end, males are introduced into captive macaque breeding groups, mimicking the natural migration patterns of these primates. However, such male introductions can be risky and unsuccessful. The procedure developed by the Biomedical Primate Research Centre (BPRC), Rijswijk, the Netherlands, to introduce male rhesus macaques (*Macaca mulatta*) into naturalistic social groups without a breeding male achieves relatively high success rates. Males are stepwise familiarized with and introduced to their new group, while all interactions between the new male and the resident females

Research Areas

General Primate Biology & Welfare

are closely monitored. Monitoring the behaviour of the resident females and their new male during all stages of the introduction provides crucial information as to whether or not it is safe to proceed. Flow diagrams identify which decisions must be made during the introduction period. The BPRC introduction procedure is widely applicable to primates with natural male migration. Following this procedure may improve the management of captive primate groups in any housing facility worldwide. Altogether, careful introduction management can minimize the risk associated with male introductions and enhance the welfare of captive primates.

Read more >

Research Areas
Tuberculosis

Tuberculosis (TB) is a bacterial infection that causes lung disease. TB is still one of the deadliest infectious disease by a single pathogen in the world. For the first time in over a decade TB deaths increased in 2021 to 1.5 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.

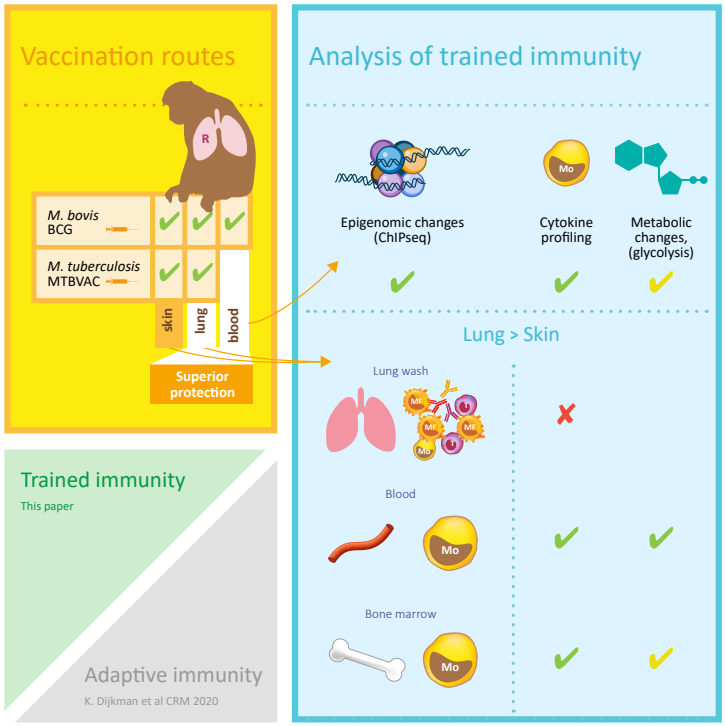
Read more >

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.



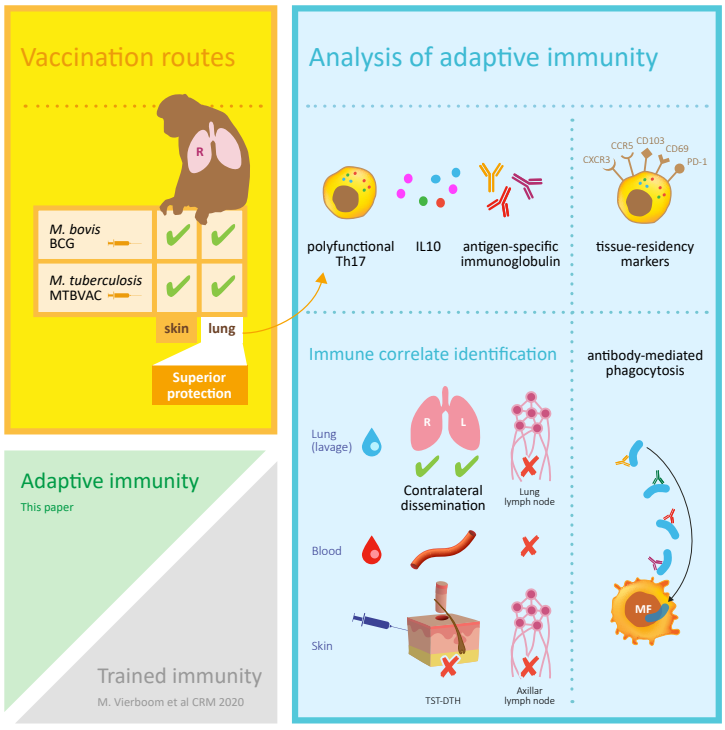
We have previously shown that mucosal vaccination with BCG delivered in the lung provided better protection against TB than classical immunization in the skin and that this protection was associated with unique TB specific local adaptive immune responses.

Read more > article and commentary.

Research Areas
Tuberculosis

We extended our analyses of the immune response observed after mucosal vaccination with BCG. This was in 2021 in Cell Reports Medicine.

Read more >



We showed for the first time in nonhuman primates that we can recapitulate the trained immunity phenotype observed in human 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 >

In addition, we analyzed the new vaccine candidate MTBVAC that was derived form a human clinical isolate of mycobacterium which is different from BCG which is derived from bovine mycobacterium.

We demonstrated that mucosal vaccination with MTBVAC also displays the same improved induction of trained immunity combined with a broadened immune response and a similar adaptive immune signature associated with the improved protection after mucosal BCG vaccination.

Read more >

Research Areas
Tuberculosis

These results provide support to strategies for improving TB vaccination and, more broadly, modulating innate immunity via mucosal surfaces.

This 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%.

We established that mucosal BCG revaccination after a standard intradermal BCG vaccination provided the same unique local immune signature as was observed with mucosal vaccination in naïve animals in association with protection against TB disease and infection. We are currently in the process of establishing the protective efficacy of this mucosal revaccination strategy.

Research Areas

Comparative Genetics & Refinement

The immune system is orchestrated by many different genes. 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 prevents 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 gene systems. A successful immune response is multifactorial, and depends, for instance, on the cooperation between the KIR and MHC system. In general, the MHC system is involved in discriminating between self and non-self and thus the recognition of invading pathogens while the KIR and LILR systems may be seen as fine tuning and serve as a correction mechanism for the MHC system. KIR genes are involved in the immune defence to viruses and cancer cells, while LILR genes play a role as modulators of infection and immunity and are engaged in neural function.

Monkeys are genetically similar to humans. Understanding genetic polymorphisms in monkeys, and their role in 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 genes from different monkey and ape species. For this, we use DNA-sequencing and other 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 Cas9 enrichment and nanopore sequencing to rapidly characterize the complex killer cell immunoglobulin-like receptor (KIR) region

The development of long-read sequencing platforms, commercialized by PacBio and Oxford Nanopore Technologies, improved the characterization of complete genome sequences. Even complex regions, represented by multigenic immune clusters and repetitive DNA stretches, are defined using these single-molecule real-time (SMRT) sequencing approaches. A correct annotation of these regions is, however, hampered by a limited coverage obtained by whole genome sequencing. For example, the major histocompatibility complex (MHC) and killer cell immunoglobulin-like receptor (KIR) immune clusters are not resolved at an allele level resolution in most genome assemblies.

Instead of sequencing the whole genome, regions of interest can be enriched to specifically improve their coverage. We adapted a Cas9-mediated enrichment approach using target-specific guiding RNAs in combination with Oxford Nanopore sequencing, to obtain long overlapping DNA fragments (7 to 22 kb) that span the multigenic KIR region. The strategy was validated by the characterization of six human and six rhesus macaque KIR haplotypes at an allele level resolution. As no amplification steps are involved, epigenetic information is maintained, and allowed methylation profiling of the clusters as well.

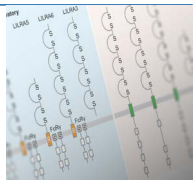
This enrichment strategy is applicable to complex regions of interest in different species, reaches allele level resolution, is free of PCR-induced errors, and is able to define epigenetic footprints.

Read more >

Research Areas

Comparative Genetics & Refinement

The primate leucocyte immunoglobulin-like receptor (LILR) region shows a high degree of conservation in the genomic organisation



The leucocyte immunoglobulin-like receptor (*LILR*) region contains several genes encoding for either inhibitory or activating receptors that are expressed by cells of the myeloid and lymphoid lineage and involved in the regulation of immune responses. For humans it is described that several LILR receptors can interact with MHC class I molecules. In non-human primates, literature on research to these types of receptors is only scarce.

Recently, genomes of several different non-human primate species have been sequenced using next-generation sequencing techniques and are made available in public databases. The *LILR* region for these species is annotated, and this allowed us to undertake a comprehensive comparison with the human *LILR* region.

The comparison revealed that the human and non-human primate *LILR* region remained largely conserved. However, an exception was provided by the common marmoset, a New World monkey species, which has its natural habitat in the forests of Brazil. A substantial contraction of the number of *LILR* genes in the centromeric as well as telomeric region was encountered in this species. In addition, a killer-cell immunoglobulin-like receptor gene, named *KIR3DX1*, mapping to the LILR region, features one copy in humans and great apes. A second copy, likely introduced by a duplication event, was observed in lesser apes and Old and New World monkey species.

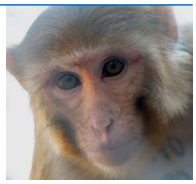
In conclusion, the data illustrates that the *LILR* gene organization shows a high degree of conservation during primate evolution and suggests that the receptors encoded by these *LILR* genes might fulfil a preserved function.

Read more >

Research Areas

Comparative Genetics & Refinement

Two human monoclonal HLA-reactive antibodies cross-react with the rhesus macaque MHC class I molecule Mamu-B*008, which is associated with control of SIV



Rhesus macaques (*Macaca mulatta, Mamu*) are often applied as pre-clinical model in transplantation research as well as to evaluate the safety and efficacy of vaccine candidates. In both lines of research, major histocompatibility complex class (MHC) I molecules play a pivotal role in the immunological responses that might be evoked. As such, the availability of non-human primate MHC-reactive monoclonal antibodies (mAbs) may enable in vitro monitoring and detection of presence of specific Mamu molecules and benefit the pre-clinical application and refinement of the rhesus macaque model.

Up till now, only one rhesus macaque MHC class I-specific mAb, detecting Mamu-A1*001:01, is available. We have screened a panel of thoroughly characterized human MHC class I-specific mAbs for cross-reactivity with rhesus macaque MHC class I allotypes using a collection of single-antigen expressing cell lines. This screening revealed two mAbs that recognize a specific epitope present on the rhesus macaque Mamu-B*008:01 allotype, which is known to be associated with elite control of SIV replication. In addition, a third mAb was found that exhibited a more complex reaction pattern and showed cross-reactivity with Mamu-A2*05:01 and-B*001:01.

Our search to expand the non-human primate mAb-toolkit resulted in the discovery of three HLA-reactive mAbs that show cross-reactivity with certain Mamu molecules and these can be applied to in vitro monitor the presence of the relevant allelic products. Moreover, the two mAbs that cross-react with Mamu-B*008:01 can be powerful mAbs for application in SIV-related research.

Read more >

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 >

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.

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

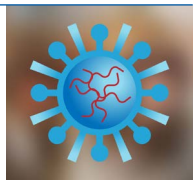
Experimental animal models for universal influenza vaccines

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

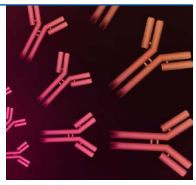
Experimental animal models for bird flu



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.

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.

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.

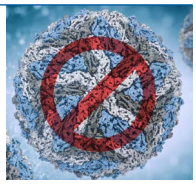
So far, vaccines are only available for dengue virus and yellow fever virus, but these vaccines have severe limitations and are not advised to people that run the highest risk for complications, namely children, older people and those people with an impaired immune system.

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. The first part of this research (effectiveness against DENV-2) will be published in 2022.

Research Areas

Mosquito-borne Diseases

A new zika virus vaccine tested in rhesus macaques

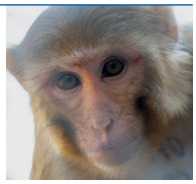
The classic method of vaccine production is time consuming, requires batch-to-batch evaluation and distribution using a cold-chain. This hampers a rapid response in case of an outbreak. The 2015-2016 outbreak of Zika virus brought the weaknesses of classic vaccines to light.

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 rhesus macaques

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. A manuscript describing the results will be published in 2022.

Research Areas

Mosquito-borne Diseases

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.

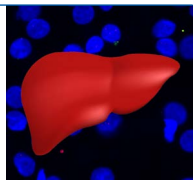
In the last quarter of 2021, we evaluated the immunogenicity of two rabies virus vaccines which were built on the backbones of the attenuated yellow fever virus vaccine strain, YFV-17D, and the Japanese encephalitis virus vaccine strain (CD-JEVAX). The first analyses show that both vaccines effectively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

Photo: Pixabay; Public Domain Pictures

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* to study hypnozoites and discover new drugs that are urgently needed to kill hypnozoites. For this we have developed an *in vitro* liver stage culture system for *P. cynomolgi.*

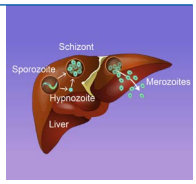
A new approach to identify drugs that can kill dormant liver stage parasites



We routinely use in vitro cultured liver-cells that are infected with *P. cynomolgi* malaria parasites to determine whether dormant liver stages are sensitive to potential antimalarial drugs. This can be done in several ways, up until now we stained the parasites with a parasite-specific reagent and counted the parasites using an automated microscope. In this way we could see whether parasites 'disappeared' from the culture after treatment with a potential antimalarial drug. To increase the through-put we have developed an assay in which parasites are directly visible, without staining (so-called bioluminescence) to measure drug activity against hypnozoites. This assay is faster, sensitive and can be used for high through-put drug screening. We have validated and optimized the method and together with a partner laboratory we have tested over 6000 compounds for their ability to kill dormant liver stage parasites. So far without finding active compounds, but the method works and is ready to be implemented in large compound screening efforts.

Research Areas
Malaria

Single-cell isolation of dormant malaria parasites

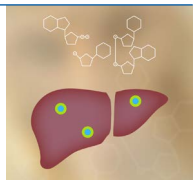


Little is known of the biology of dormant malaria parasites that reside inside the liver, so-called hypnozoites. We use the monkey malaria Plasmodium cynomolgi as model system to investigate this enigmatic stage of the parasite life cycle. We have developed in vitro culture methods for growing both dormant and developing liver stage parasites in liver cells. Unfortunately, only a minor proportion of liver cells becomes infected with parasites in vitro. This complicates investigations into which genes are expressed in these stages.

To overcome this, we have developed transgenic parasites that express the fluorescent marker green fluorescent protein (GFP). This enabled fluorescent activated cell sorting (FACS) methods for the isolation of dormant and developing liver stage parasites for further analysis. We have published this method in 2021. To enable a more detailed analysis of gene expression in dormant parasites, we have further developed this FACS method. We are now able to isolate single parasites and characterize their gene-expression pattern. By doing this, we hope to gain a better understanding of the mechanisms underlying liver stage parasite dormancy.

Research Areas
Malaria

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



In 2021 we carried out a study, funded by GHIT Fund (Global Health Innovative Technology Fund) with the purpose of developing a rapid test for identifying people that are “latently” infected with malaria.

The project is composed of two connected animal experiments: the first was aimed at identifying unique metabolic signatures in the serum of animals “latently” carrying dormant malaria infection stages (hypnozoites). The second phase will be aimed at determining the sensitivity of the metabolite approach *in vivo*.

The set up for this experiment was as follows: Two groups of five rhesus macaques were infected with a high dose of *P. cynomolgi* parasites (sporozoites), forming the dormant stage of malaria (hypnozoites), and one group of five rhesus macaques with *P. knowlesi*, a non-human primate and human parasite that does not form hypnozoites. One of the two *P. cynomolgi*-infected groups was prophylactically treated with atovaquone, to kill the active parasites (growing liver states) resulting in an infection with dormant stage hypnozoites only, that would relapse around day 21. The *P. knowlesi* group was also treated with atovaquone to eliminate all liver stages and serve as control. Primary parasitemia in the other *P. cynomolgi*-infected group was cured with chloroquine. Serum from all animals was regularly sampled for metabolomic analysis from day 0 until the first relapse was apparent, *P. cynomolgi* groups were then cured with anti-malarial drugs tafenoquine and chloroquine.

We identified approximately 40 metabolites in the serum that were selectively expressed in the dormant stage (hypnozoite) condition only. Now we have identified these 40 metabolites we can proceed to the second part of the experiment of establishing the sensitivity of detection, which will be carried out in 2022.

Research Areas

Neurobiology & Aging

This year a new department started under the name Neurobiology & Aging. This department will work on age-related neurodegenerative diseases such as dementia and Parkinson's disease, but also on the effect of normal aging on the brain. Areas of focus for this department are neuroinflammation, neurodegeneration and peripheral-central interactions, like the effect of SARS-CoV2 infection on the brain. In 2021, two PhD students started in the department Neurobiology & Aging to work on these projects.

Aging as risk factor for neurodegenerative diseases

Aging is a major risk factor for neurodegenerative disease, and with a growing elderly population, its prevalence is continuously increasing. However, it remains a mystery why largely elderly are affected. The normal aging process in the healthy brain is associated with a decline in physiological function, an increase in neuroinflammation, brain shrinkage, and memory deficits. Previous research in mice has shown that factors present in old blood can impair synaptic plasticity and cause memory impairment, processes that are similarly affected in Alzheimer's disease for example. Vice versa, young blood proteins can rejuvenate several organs in aged mice, including the brain, and have also shown to reverse synaptic changes and memory impairment in a mouse model for Alzheimer's disease. Therefore, we aim to elucidate which blood factors are altered with aging and could negatively affect several aspects of neurodegeneration, which could lead to new interventions to stop or even prevent or treat such diseases.

One hallmark that is shared by many neurodegenerative diseases is neuroinflammation, which is also increased with advancing age. Neuroinflammation can occur through the activation of glial cells like microglia and astrocytes, and through cells of the innate and adaptive peripheral immune system.

Research Areas

Neurobiology & Aging

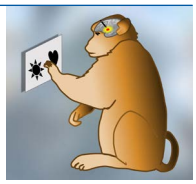
Although we know aging is a key contributor to the progression and aggravation of neurodegenerative diseases, it is challenging to determine their exact relationship. By analysing brain samples of different species and different ages, and their blood factors, including proteins and immune cells, we aim 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.

The results presented here from 2021 are from research projects that already started before the installation of this new department.

Research Areas

Neurobiology & Aging

Deep Brain Stimulation (DBS) in Parkinson's disease



With approximately 7 million people worldwide suffering from Parkinson's disease (PD), it is one of the most common age-related diseases of the central nervous system. PD is caused by the death of neurons in a part of the brain that controls movement. This damage results in the typical motoric symptoms, such as tremors, rigidity, or slowness of movement. Other symptoms include sleep disturbance, depression, and dementia. PD dementia (PDD) is one of the most disabling symptoms of PD. This type of dementia is caused by degeneration of neurons in the nucleus Basalis of Meynert (NBM) which controls cognition through the innervation of the cerebral cortex with a messenger compound acetylcholine.

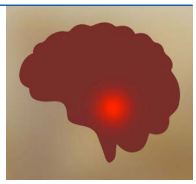
In collaboration with the University Medical Center Groningen, a model was developed in the rhesus monkey for PDD, to ultimately study the role of deep brain stimulation (DBS), a therapy most used to treat motor symptoms, in improving cognitive impairment in PDD. In this new animal model, cognitive decline is modeled by a chemically induced lesion of cholinergic neurons in the NBM under guidance of MRI and CT brain scans. The cognitive changes in memory are tested in the home-cage with a touchscreen tablet. The monkeys learn to use the tablet to perform cognitive tasks. The next step is to validate the lesion in the NBM and to investigated whether this led to impaired performance on the cognitive tasks.

Read more >

Research Areas

Neurobiology & Aging

SARS-CoV-2 induced brain inflammation and Parkinson-like neuropathology



Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes a multi-system inflammatory disease syndrome known as COVID-19. Besides the common effects on the respiratory system, the SARS-CoV-2 infection may also cause neurological complications in infected humans. At the BPRC we investigated the impact of SARS-CoV-2 on the brains of four rhesus and four cynomolgus macaques. Longitudinal positron emission tomography and computer tomography (PET-CT) documented possible inflammation in the brains of infected individuals of both species. Even 30 days after viral clearance from nasal and respiratory tract, postmortem brain analysis confirmed infiltration of both T-cells and activated microglia at various brain sites in all infected monkeys indicating inflammation in the brain, which was not observed in control animals. In one animal, SARS-CoV-2 viral RNA was detected postmortem in several brain areas. Notably, in the midbrain of all infected rhesus macaques, α-synuclein aggregates were observed, similar to Lewy bodies, which in humans are indicative of the development of Parkinson's disease. These findings represent a warning sign of potential long-term neurological effects following SARS-CoV-2 infections. These findings underscore the neuropathological potential of SARS-CoV-2 and emphasize the virus' capability to induce neuroinflammation and α-synuclein aggregation, in an important nonhuman primate model for human disease.

Read more >

In 2021, the delta variant of SARS-CoV-2 became the predominant variant of concern. Another study started at BPRC in which 4 resus macaques were infected with this variant. These monkeys were imaged longitudinally with a PET-CT tracer more specific to neuroinflammation, namely [18F]DPA-714, which targets the translocator protein TSPO. Ongoing research is further investigating neuroinflammation and neuropathology in postmortem brain regions of these animals and of age-matched non-infected controls.

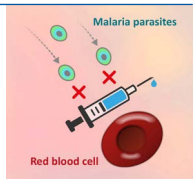
Research Areas

Protein Core Facility

BPRC is often a collaborative partner to evaluate new vaccine candidates. Yet, we also design vaccines ourselves. Scientists at BPRC have many years of experience in vaccine development.

COVID vaccine

BPRC is in the process to produce an attenuated virus vaccine against COVID19. Using Transformation Assisted Recombination techniques, hybrid common cold-SARS CoV2 coronaviruses are being constructed, among others with the delta variant of the virus. When available, these will be tested for safety and efficacy as a potential superior vaccine. Moreover, they may serve as research tools to elucidate the mechanisms by which monkeys, and also humans, protect themselves against COVID.



Malaria vaccine

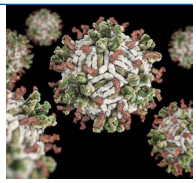
PfAMA1, a protein vaccine against the deadly malaria parasite *Plasmodium falciparum* was developed at BPRC's protein core facility. AMA1 plays a key role in the entry of the parasite into red blood cells and therefore AMA1 is a good vaccine candidate. After optimizing vaccine design, expression and purification procedures the vaccine was used in a proof-of-concept study in monkeys. After an extended vaccination procedure PfAMA1 provided partial protection against the malaria disease. However, in people the vaccine did not suffice. Combining PfAMA1 with another vaccine candidate may overcome this in the future.

Research Areas

Protein Core Facility

The AMA1 protein from the second-most important human malaria, *Plasmodium vivax*, has also been produced and is being tested as a potential vaccine. First results show that high levels of antibodies are induced after vaccination that prevent invasion in red blood cells in functionality tests in the lab. Tests in humans are intended, but external funding needs to be acquired for this purpose.

Virus like particles vaccine platform



Virus-like particles (VLPs) are molecules that resemble viruses but lack the virus' genetic material, and thus are non-infectious. VLPs are capable of activating the immune system and generate a virus-specific immune response. Therefore, VLPs are sometimes used as a vaccine. The vaccine that is currently used to protect women from cervical cancer caused by HPV is also based in virus like particle technique.

In line with this, BPRC's protein core facility designed VLP-vaccines for Usutu virus and Zika virus. Both vaccine candidates are in early stage of development. The envelope proteins were produced by recombinant techniques, purified and biochemically prepared to generate VLP's. Vaccination studies showed that antibodies obtained after vaccination with usutu or ZIka protein fractions prevent virus replication in functionality tests in the lab. However, the proteins need to be adapted to optimize VLP formation. After that, they will be further evaluated for immunogenicity and their potential as human vaccines.

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

61 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 gave an interview to the Volkskrant resulting in

an international award winning article

on two monkeys that were followed during a COVID experiment.

We actively

feed our website

with relevant updates.

We organized

bachelor, master & PhD courses

for future scientists.

During

(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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BPRC Annual Scientific Report 2021
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Biomedical Primate
Research Centre