	Inventaris Wob-verzoek W17-12								
		wordt verstrekt				**weigeringsgronden**			
nr.	**documenten NTS20172044**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel				x		x	x	
3	Niet-technische samenvatting	x							
4	Bijlage beschrijving dierproeven 1				x		x	x	
5	Bijlage beschrijving dierproeven 2				x		x	x	
6	Bijlage beschrijving dierproeven 3				x		x	x	
7	Bijlage beschrijving dierproeven 4				x		x	x	
8	DEC-advies				x		x	x	
9	Ontvangstbevestiging				x		x	x	
10	Advies CCD		x						x
11	Beschikking en vergunning				x		x	x	

2044

1.

Centrale Commissie Dierproeven

0 9 JUNI 2017

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in*	11400
☐ Nee > *U kunt geen aanvraag doen*	

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	Vrije Universiteit Medisch Centrum (VUmc) te Amsterdam	
Naam van de portefeuillehouder of diens gemachtigde	[redacted]	
KvK-nummer	64156338	
Straat en huisnummer	de Boelelaan	1117
Postbus		
Postcode en plaats	1081HV	Amsterdam
IBAN		
Tenaamstelling van het rekeningnummer		

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted]	☐ Dhr. ☒ **Mw.**
Functie	[redacted]	
Afdeling	[redacted]	
Telefoonnummer		
E-mailadres		

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted]	☐ Dhr. ☒ **Mw.**
Functie	[redacted]	
Afdeling		
Telefoonnummer		
E-mailadres		

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	Is er voor deze projectaanvraag een gemachtigde?	☒ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☐ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 oktober 2017
		Einddatum	30 september 2022
3.2	Wat is de titel van het project?	Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration.	
3.3	Wat is de titel van de niet-technische samenvatting?	De experimentele therapeutische transplantatie van stamcel-retinaal pigment epitheel in diermodellen voor retinale degeneratie.	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC Vrije Universiteit / VU Medisch Centrum
		Postadres	[redacted] [redacted] Amsterdam | Nederland
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

☒ Nieuwe aanvraag Projectvergunning € 1684 Lege
☐ Wijziging € Lege
☐ Via een eenmalige incasso
☒ Na ontvangst van de factuur*

Factuur graag opsturen naar:
[redacted]

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht
☒ Projectvoorstel
☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing
☒ Melding Machtiging
☐

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam	[redacted]
Functie	
Plaats	Amsterdam
Datum	07 - 06 - 2017
Handtekening	[redacted]

2.

Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11400
1.2	Provide the name of the licenced establishment.	VUmc
1.3	Provide the title of the project.	Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration

2 Categories

2.1	Please tick each of the following boxes that applies to your project.	X Basic research
		X Translational or applied research
		☐ Regulatory use or routine production
		☐ Research into environmental protection in the interest of human or
		☐ Research aimed at preserving the species subjected to procedures
		☐ Higher education or training
		☐ Forensic enquiries
		☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Background information

Genetic blindness.

Millions of people are estimated to be visually impaired worldwide. In the Netherlands, about 50,000 people are affected with hereditary blindness caused by a single disease gene such as Retinitis Pigmentosa (RP). Over 350,000 people are affected with a more complex, visual disability such as age-related macular degeneration (AMD), where both multiple genes and environmental factors play a role.

The retinal pigment epithelium (RPE).
The RPE is a multi-functional neuro-epithelial cell layer located between the photoreceptors (rods and cones) and the choroid (the blood vessels). The RPE ensures that the visual cycle remains active and forms the barrier between the choroidal blood vessels and the cell layers of the retina. In summary, the RPE layer is essential for visual function.

Disorders of the RPE: Retinitis Pigmentosa (RP) and age-related macular degeneration (AMD).
RP (or tunnel vision) is a hereditary progressive retinal disease, with a frequency of 1 in 4,000 humans. It mainly affects children or young adults. A subset of patients have a mutation in a gene with RPE-specific expression, [redacted]. Biochemically, both genes play a role in feeding and maintaining the signal transduction cascade in the retina. Local oxidative stress in the RPE, [redacted] degeneration.

AMD is a progressive degenerative retinal disease, which affects 4% of the population over 60, and 15% above 70 years of age. Early AMD is characterized by the development of so-called drusen (abnormally accumulating metabolic waste material) under the RPE cell layer and a slowly less functioning RPE. In the later stage, AMD can be divided in geographic atrophy (dry AMD), or neovascularization of the choroid (wet AMD). The dry form of AMD is characterized by the presence of drusen (at the macula) and a slowly deteriorating RPE function. The wet form of AMD occurs in only 10% of all AMD patients and is characterized by extensive growth of new leaky choroidal blood vessels, which disrupt the blood-retina barrier and damage the retinal neural tissue.
Both environmental factors such as age, smoking and diet and genetic factors affect the disease. Recent genetic genome-wide association studies indicate at least nineteen candidate AMD genes.

Experimental treatments of disorders of the RPE: RP and AMD
After obtaining proof-of principle in mice, RPE65 gene therapy clinical trials are currently ongoing in the form of *in vivo* gene therapy, and dietary vitamin A-derivative supplementation. However, both experimental treatments currently suffer from side effects and drawbacks: While initially successful, intra-ocular injections of a relevant adeno-associated virus, RPE65 gene therapy resulted in only approximately 60-70% transduction of cells in the target area. The remainder of the cells is either killed, not transduced, not cured and continues to degenerate. The systemic supplement of retinoids in clinical trials have been controversial given their potential toxicity. Indeed, in the ongoing clinical trial with a chemically modified oral retinoid in RPE65 patients, patients suffer from severe headaches. [redacted]
Currently, there is no effective or fully approved treatment for the most common (dry) form of AMD. For the wet form (two-) monthly intra-ocular injections with anti-VEGF medication, photodynamic therapy and laser treatments are frequently given to prevent (further) neovascularization. The effectiveness of the treatments vary between patients.

A new development: [redacted] *degenerative diseases of the RPE*
As summarized above, all relevant current RPE treatments of RP and AMD, except for the rare wet AMD type, are ineffective, patient-unfriendly and mostly require living cells. Sometimes, they prevent further loss of vision. **However, once RPE cells (and vision) are lost, there is no alternative to replace the RPE.** These experimental transplantations of the RPE are inefficient, mainly due to limited availability of suitable (autologous) donor material. [redacted]
Previously, at least two research groups reported some functional visual improvement in (blind) mice or rats [redacted]

Our preliminary work
Over the last years the research of our group focused [redacted]

[redacted]
foreign work visits.
In our lab we set up and gained experience with a brand-new, noninvasive, ocular screenings facility for small animals. This includes optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO) and Electroretinograms (ERG) screenings. We also set up an ocular injection and surgery unit for mice and rats. All these techniques and facilities are now operational and form a single pipeline [redacted]

[redacted]

[redacted] to human clinical trials.
We are convinced that there is no other solution than the use of animals for such experiments. The use of our non-invasive screening facility minimizes the number of animals used and animal discomfort as much as possible.

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The overall aim of our project is [redacted] in suitable animal models of retinal degeneration, in order to gain a much more prolonged and improved functional recovery (>50%). We recently showed that the molecular signature of *in vivo* [redacted] shows substantial differences, but we do not know whether this has also functional consequences. In the literature and our hands, injected dissociated [redacted] such as some types of RP, and AMD.

The main aim of [redacted] RPE cells in animal models for retinal degenerative disease in order to gain an improved functional recovery. A number of sub-aims support this main aim:

1. What is the exact onset and progression of visual impairment in our non-treated AMD animal models compared to normal vision in wild type controls (rat, mice)?
2. What is the onset and progression of visual impairment in our treated animal models compared to not-treated animals?
3. Is our [redacted] safe and efficient over a prolonged period of time?
4. Can we combine our [redacted]
5. Can we use our [redacted]

[redacted]

We will measure and follow-up the onset and progression of retinal degeneration in our non-treated [redacted] models non-invasively by OCT, SLO and ERG measurements. These examinations are essential to establish a baseline for retinal degeneration in our treated and not-treated animal models.

With the investigation of these research questions we aim to set an essential step towards the use [redacted] trials in AMD and some forms of RP.

The feasibility of the project
This project is a logical progression with a refinement and improvement of our own experiments and those already published research in the peer reviewed literature. With the injection of single RPE cell suspensions in a mouse or rat model, variable rates of visual improvement will be observed, as measured by ERG and immunohistochemistry. [redacted]

We have sufficient manpower, expertise and resources to carry out this project successfully. We recently installed a new facility to screen small animals non-invasively by OCT, SLO and ERG. This technology allows us to follow the animals over time and to measure the visual activity of the animals. Over the past few years we have gained the skills to operate these machines and mastered [redacted]

[redacted]

This research plan has been scientifically examined and our expertise is visible in the articles which are published in several scientific journals. Apart from our own expertise we have made highly relevant work visits to/collaborations. We also followed several courses, such as SLO/OCT, ERG handling and mouse microsurgery.

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

The scientific importance consists mainly of further investigations of the molecular and functional characteristics of the RPE and the retina. We will also gradually learn much about interaction of RPE with photoreceptors (and retina), the [redacted] will open up a lot of possibilities to study possible treatments of retinal degeneration similar diseases.

The social importance is, as mentioned above, huge. The Netherlands have about 50,000 people with monogenic hereditary blindness and 350,000 people with a more complex form of genetically determined blindness. Monogenic blindness (caused by a mutation in one gene), such as RP, mainly affects children, who suffer from their handicap for their entire life. Complex blindness, such as AMD, severely affects the quality of life of older people and they will therefore lose progressively a significant proportion of their autonomy. Up to now there is no effective, efficient and long-lasting treatment for both diseases. Our proposed transplantation strategy might provide one.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

[redacted]. After transplantation, we will compare the onset and progression of disease and rate of recovery among the different approaches, with the aim to select the best experimental therapeutic strategy.

[redacted] We will next follow the onset and progression of pathology non-invasively by OCT, SLO and ERG, and eventually immunohistochemistry. [redacted]

[redacted]
Obviously, from the combined experiments above, we will gain a vast amount of data about potential ocular infections (despite the ocular immune privilege), uncontrolled cell growth [redacted] and success or failure rates. We will continuously assess these data to determine part of the safety and utility of this approach in concrete numbers.

For the complex disorder AMD, [redacted], in order to postpone the onset or progression of this age-related disease for 10-20 years. In contrast, disorders caused by one mutation in a single gene occur much earlier in life and [redacted].
Currently, injection gene therapy is not satisfactorily. [redacted] from RP patients with a defect in a single gene, [redacted] (available from collaborators). Next, [redacted] and we follow the onset and progression in time using Optical Coherence Tomography (OCT), Scanning Laser Ophthalmoscopy (SLO) and Electroretinograms (ERG) (please find explanations of the techniques below). [redacted]

Animal models used in this study

[redacted] This method has proven previously to be successful in larger animals, and, [redacted]. Injection with higher concentration [redacted], and invokes a currently undesirable phenotype including neovascularization. [redacted]

For our experiments it is essential to distinguish between (partial) RPE function of the host [redacted] Therefore, we will inject the [redacted] This is a host in which the [redacted] due to [redacted]
In contrast, the transplanted RPE cells do carry an active gene. Consequently, [redacted] **(2)**. This functionality of the visual cycle can be measured by ERG. In addition, safety studies will be performed in wildtype animals **(3) as a control.**

The use of the [redacted] rat provides us also with an interesting spin-off possibility. It is expected that these animals by themselves, [redacted] and only after prolonged [redacted], develop (not AMD but) RPE related photoreceptor degeneration. Gene therapy by direct ocular injection for this disorder yields limited results, most likely due to low transfection efficiency *in vivo.* [redacted] also in mice **(5)**, for which a diversity of other models for the numerous types of RP are available.

Mice and rats are ideal animal models for this type of research, because they are mammals and therefore phylogenetically closely related to humans, yet also uniquely amendable to genetic modifications. In addition, the morphology of their eye closely relates to that of humans and the eyes show functionally a high similarity.
Since the size of the rat eye is bigger than a mouse eye, the rat might be a better option to perform complex therapies which contain [redacted] However, only few transgenic rat lines exist, which can be used for ophthalmology research. In mice, quite a few genetic lines already exist. This is a major advantage of the mouse as animal model in this project. This is why we will aim for a procedure which works in rats (advantage: bigger eye, disadvantage: no broad availability of transgenic lines) and mice (advantage: multiple models, disadvantage: small eye, pilot first).
Below we list the advantages and disadvantages of rat and mouse models.

	Rat	Mouse
Advantages	• Eyes are bigger and hence more approachable • [redacted] • Data less error-prone (less failure of transfer)	• Numerous models available For both monogenic and complex disease • More relevant data available in literature • New models made routinely
Disadvantages	• Only one/two genetic modes and one chemical model available. • Less transfer data (of cell suspension transfer) available in literature. • Little routine in new model construction	• Very small eyes (less than an pea) • [redacted] techniques, if possible, need to be further established • Error rate (even with ocular injections: quite high)

Figure 1. Advantages and disadvantages of the rat and mouse models.

General methods

[redacted]
Depending on the research question asked, we will use two different methods of [redacted]: (1) [redacted]; or (2) [redacted] To investigate [redacted] are harmless for the host animals.

[redacted] ***method 1:*** [redacted]
Essentially, [redacted] We are currently looking into the possibility to improve the existing protocol of this procedure by introducing new equipment. [redacted] will be used as a control to compare the new [redacted] to.

[redacted] ***method 2:*** [redacted]
Through work visits, we recently learned [redacted] in human, rabbits and rats. [redacted] in humans and rabbits is highly comparable, and is carried out with the same instruments and methodology. Given the small size of the eye, [redacted] in the rat is more complicated. The smallest sized instruments commercially available are being used. This technique requires more handlings than [redacted] This is the case because [redacted]. In summary, [redacted]

Scanning Laser Ophthalmoscope (SLO) and Optical Coherence Tomography (OCT)
SLO and OCT are techniques which are used for scanning of the eye and retina structures, also in humans. Both are relatively new techniques, which are non-invasive and completely harmless. A few modifications to the clinical apparatus used, were made, [redacted]. In summary, a small laser beam beams through the pupil and scans the back of the eye (SLO) or it scans, layer by layer, through a section of the retina (OCT).

Electroretinogram (ERG)
Using an ERG machine one measurers, noninvasively, the response of the retina to electrical micro responses generated by the retina after flashes of light. Many layers of the retina yield a specific signal on the electroretinogram.

***In vitro* analyses**
At the end of the follow-up experiments, the animals will be sacrificed and the eyes will be enucleated. All tissues will be used for further *in vitro* analyses.

Timeline
Table 1 shows an estimated timeline for the project.

Table 1 An estimated timeline which divides the experimental procedures over five years. Breeding (appendix 1) will be done by the animal facility during Year 1-4. Year 5 will be used when experiments take more time than expected. In Year 1 we will set-up and optimize our non-invasive screening facility for mouse and rat. This includes the use of SLO, OCT, ERG and behavioral assays. Next, we will screen our animal models to answer question 1. [redacted] methods will also be set up in Year 1. We expect to start the first experiments from appendix 3 in Year 2. We will then [redacted] in rat models and follow-up all animals which are in the experiments. At the end of all experiments, the animals will be sacrificed and tissues will be harvested for in vitro analyses. Year 3 will be used for the further [redacted] in rats and the start of appendix 4 [redacted] This is followed by the [redacted] which is described in appendix 3 (in mice or rats). Year 4 will be used to finalize [redacted] and to finalize all follow-up measurements and safety.

Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Breeding	Breeding	Breeding	Breeding	Describe and publish results
Setup non-invasive screening methods for mouse and rat.	[redacted] in rat models	Introduce the mouse as a model. [redacted]	Determine safety of procedures. Follow-up of all experimental animals.	Harvest eyes and tissues and perform *in vitro* analyses
Screening of animal models.	Follow-up of the animals which are in the experiments.	[redacted]	Harvest eyes and tissues and perform *in vitro* analyses	
Set-up [redacted] in rats.	Harvest eyes and tissues and perform *in vitro* analyses.	Harvest eyes and tissues and perform *in vitro* analyses		

Within the project the following questions are addressed:

1. What is the exact onset and progression of visual impairment in our non-treated AMD animal models compared to normal vision in wild type controls (rat, mice)?
2. What is the onset and progression of visual impairment in our treated animal models compared to not-treated animals?
3. [redacted] safe and efficient over a prolonged period of time?
4. [redacted] retinal diseases of the RPE (i.e. certain types of RP) efficiently?
5. [redacted]

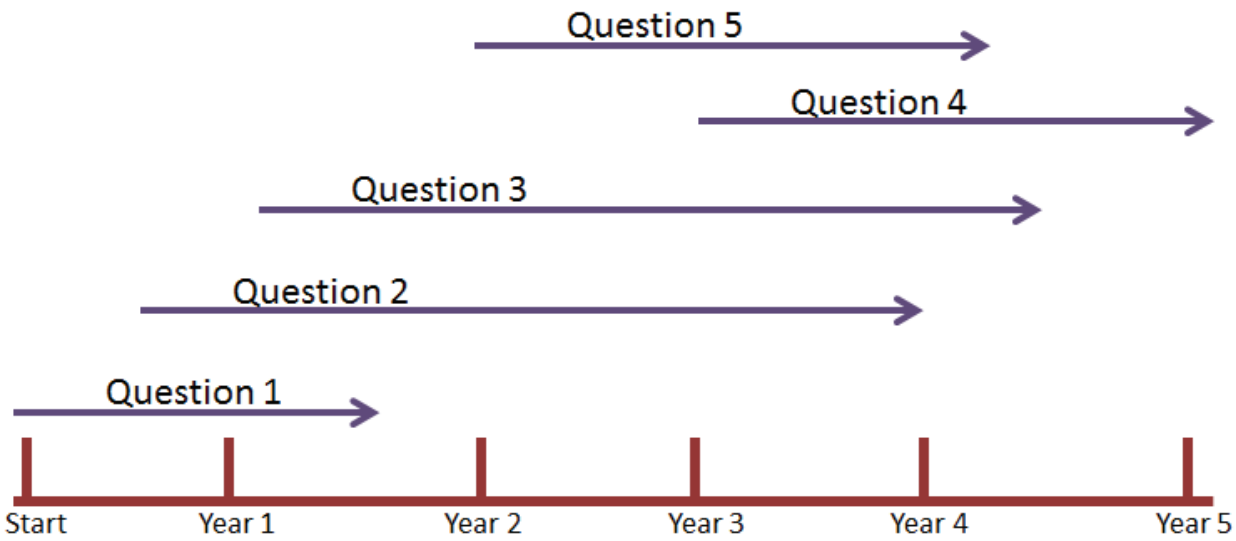


Figure 2. An estimated timeline of the project. The questions which are addressed in this project are schematically divided over the five years. Question 1 will be addressed at the beginning of the project. Our follow-up of the experimental animals which received a [redacted] will take approximately 3.5 years (question 2 and 3). Once we are confident in [redacted] (question 5; appendix 4). Once we have an answer to question 5, we will start to work on [redacted] (question 4). Preparations for this will be done on forehand.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

The experiments which are described in this document will be performed in different animal experiments described below.

Breeding and phenotyping the experimental animals (appendix 1).
We will need genetically modified animals (breeding with discomfort) for our experiments. The genes which are knocked out in these models are essential for normal vision. Therefore, the experimental animals will be (partly) blind. Because of this visual impairment, this is breeding with discomfort. The animals will be used in the experiments which are described in appendices 2-4. [redacted]. This will be done by SLO, OCT, ERG measurements and visual behavioural analyses.

[redacted] *(appendix 2).*
[redacted] is partly set-up and needs to be optimized. This is still an experimental procedure. Therefore, we will start the experiments in this appendix with animals which will be terminated after the procedure. If we are confident that the procedures will not leave any (long term) damage to the eye, the animals are allowed to wake up and the effects are examined after a few days. [redacted] is well defined, and we have sufficient experience in that area.

[redacted] *in animal models for retinal degenerative diseases and its safety (appendix 3).*
Once the [redacted] is setup in wild type healthy rat (or mouse) we will proceed to the experimental animals. These animals will have a defect in their retina. This is either caused [redacted] will be performed:
In summary, the animals will undergo baseline measurements using the non-invasive techniques SLO, OCT and ERG. [redacted] in one of the two eyes. The other eyes will serve as a control eye. The animals will be screened again by SLO, OCT and ERG a few days [redacted]. The animals will be followed with the same visual screenings techniquesfor a maximum period of a year after transplantation. We will also perform visual behavioral experiments. [redacted]

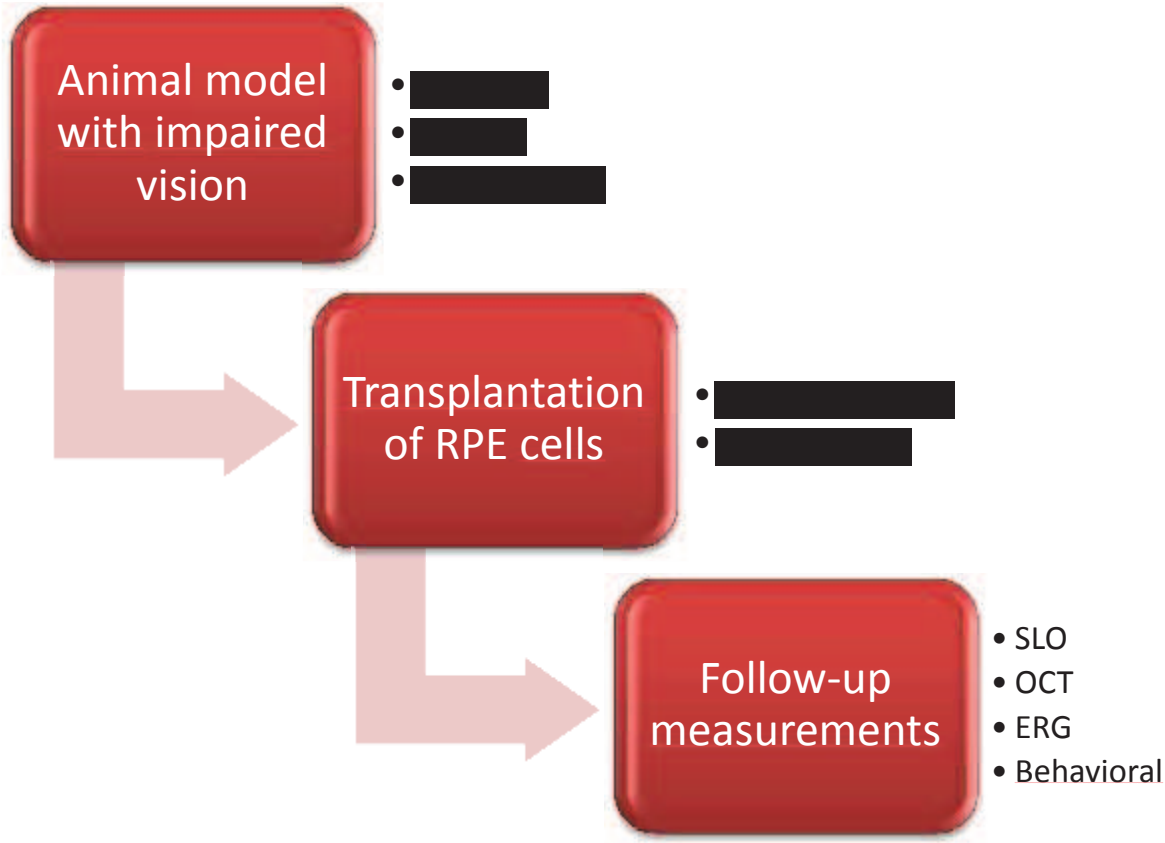


Figure 3. A flowchart of the general experimental setup. We will start with an animal model (mouse or rat) with an impaired vision [redacted] *As an intervention we will* [redacted] *After this, the animals will be followed over time with our non-invasive screening methods (SLO, OCT, ERG and behavioral assays). Appendix 1: breeding and phenotyping. For the phenotyping of the animals we will only perform "follow-up" measurements without an intervention. Appendix 2:* [redacted] *and follow-up. Same goes for appendix 4, but in that case* [redacted] *will be used. The animals from appendix 3 will undergo this entire flowchart.*

[redacted] *(set-up method) (appendix 4).* [redacted] is not yet performed [redacted] and needs to be set up. In the first place, we will use animals which will be terminated after the procedure. Once we are confident that the procedure will not leave long term damage to the animal, we will allow the animals to wake up after anaesthesia and judge within the next few days whether the [redacted] was a success. The animals will be monitored with the non-invasive techniques SLO, OCT, ERG and behavioural experiments. Once we book success, we will start performing in the first disease model. [redacted]. We will practice this technique a few times and reach a success rate of at least 60%. We will expect little complications with this technique.

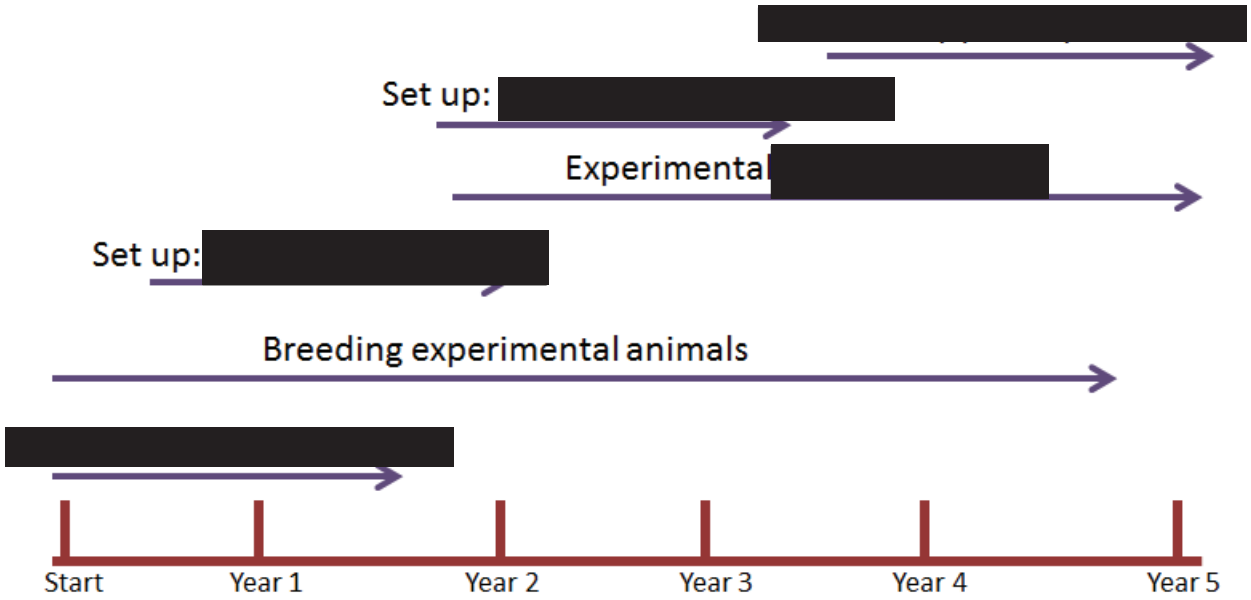


Figure 4. An estimated timeline of the project. The [redacted] (appendix 1) will be done from the start of the project. We expect to breed animals over almost the entire course of the project (appendix 1). The set-up of the [redacted] (appendix 2) will start, shortly after the start of the [redacted] Just before the start of year 2, we expect to start the experimental [redacted] (appendix 3). Simultaneously we will explore the possibility of the use of mice (appendix 4). Once we know whether mice are a possibility, we will start [redacted] (question 4, appendix 3).

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

All the above mentioned experiments serve a common goal: to [redacted]. We use different sources [redacted] and quality of visual behavior in different animal models as primary outcomes. In order to monitor the efficacy of the phenotype as a whole, as well as in sub phenotypes (remember, AMD is a heterogeneous disease!). Unfortunately, only a few (genetic) phenotypes are currently available in rats, while several disease models with relevant sub phenotypes are available in mice.
The experiments will result in a "best" procedure for [redacted] in AMD, which, eventually, can be tested in the next phase: human clinical trials. Prior to this project, we have performed enough research to take this next step. We think that the project will obtain enough important information to be approved. We are convinced that we can answer the questions, which are described in all sections above, within the given five years of this project.

Go/no-go moments
Since this project involves an experimental [redacted] procedure, clear go/no-go moments are built into the pipeline. After each answered research question we will check whether the next one is still relevant. The research strategy will be adapted if necessary, depending on the results obtained. Obviously, if, over time an experiment is not relevant anymore it will not be carried out.

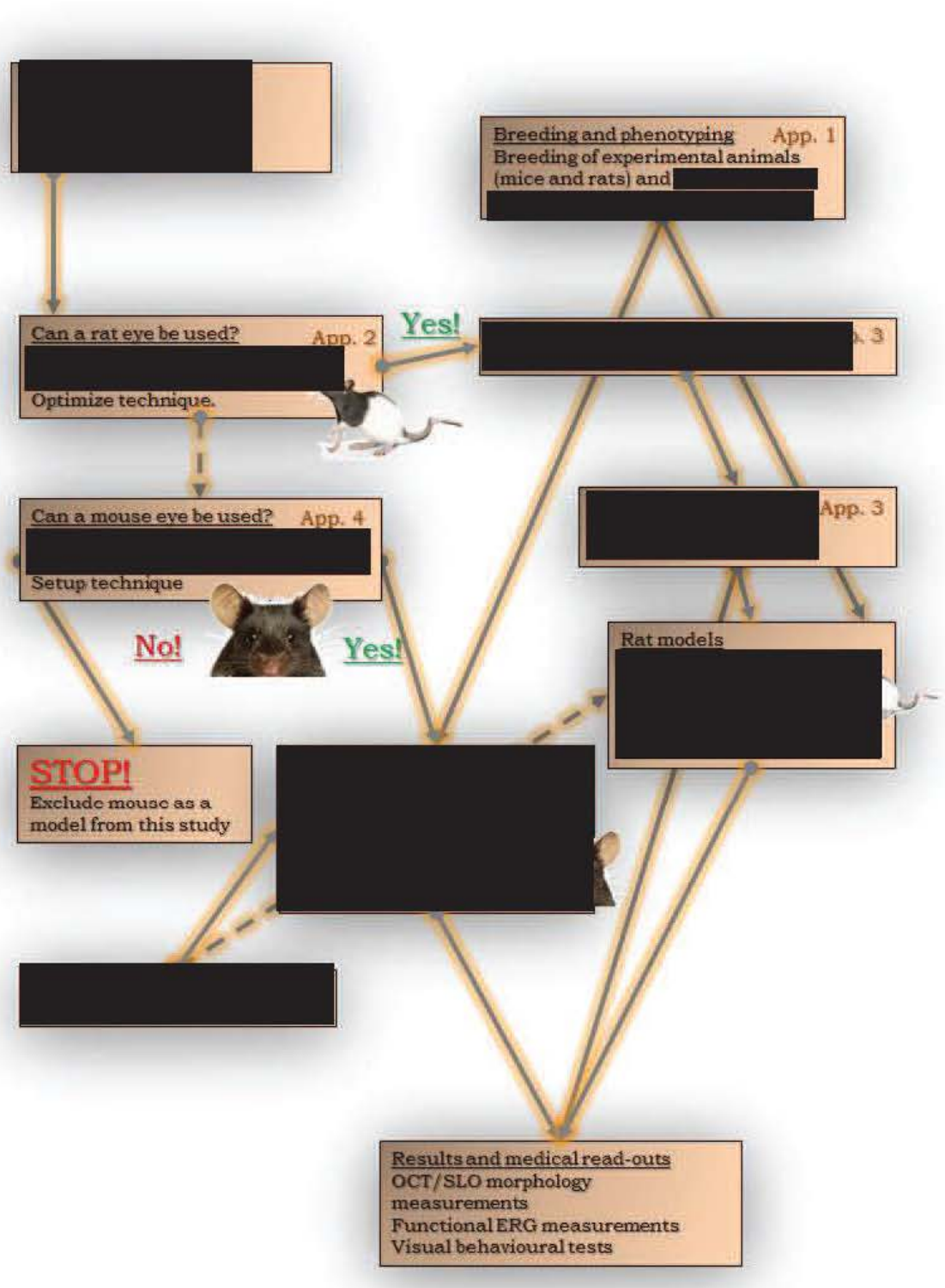


Figure 5. Flowchart stating our go and no-go moments. We will start our [redacted]. We expect that the [redacted] is technically possible (appendix 2). Once we find the best [redacted], we will compare [redacted] Our [redacted] model will be used, [redacted] and [redacted] will be used. All results will be in the form of OCT, SLO and ERG measurements and behavioral assays. We will start

examining whether the mouse is a possibility for our transplantation procedure once we have sufficient experience in the rat. This pilot will have a clear GO or NO GO moment. If we don't succeed we will exclude the mouse from our studies. If we do succeed we will test [redacted] *We will obtain the same read-outs as we do for rats. Meanwhile, we will perform our* [redacted] *in either mouse or rat depending on the outcome of appendix 4.*

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Breeding and phenotyping the experimental animals.
2	[redacted] procedure [redacted]
3	[redacted] for retinal degenerative diseases and its safety.
4	PILOT: [redacted] (set-up method).
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

4.

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11400
1.2	Provide the name of the licenced establishment.	VUmc
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Breeding with discomfort and phenotyping

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Primary goal

The primary goals of this appendix can be divided into two parts: to breed all needed genetically modified animals (mice and rats) for experiments and to [redacted] which is created.

Breeding

The aim of this procedure is to breed sufficient numbers of animals for the planned experiments (which are described in other appendices) and [redacted].
The breeding of animals with a chance of discomfort from their genetic modifications will be described here. The only animals with a chance of genotype-related discomfort are homozygous offspring of heterozygote – heterozygote crossings. The homozygous mutants will develop blindness within a few months.

[redacted] will be determined, including:

[redacted]

[redacted]

These screening methods will also be used for the experimental animals to test effectivity after intervention (other appendices).

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Breeding

Animals will be group housed and mated according to well established experimental housing procedures for animal breeding. To achieve the best protection from possible contamination, the animals will be kept in a barrier environment such as individually ventilated cages (IVCs), fed sterilized chow food and only handled under a flow hood.

Phenotyping

SLO-OCT and ERG measurements will be both performed within the timeframe of one anaesthesia. These non-invasive techniques are used to follow the morphology and function of the retina over time within the same animal. We expect to measure each animal at least three times within the timeframe of one year. The animals will be in experiments for a time period of one year.

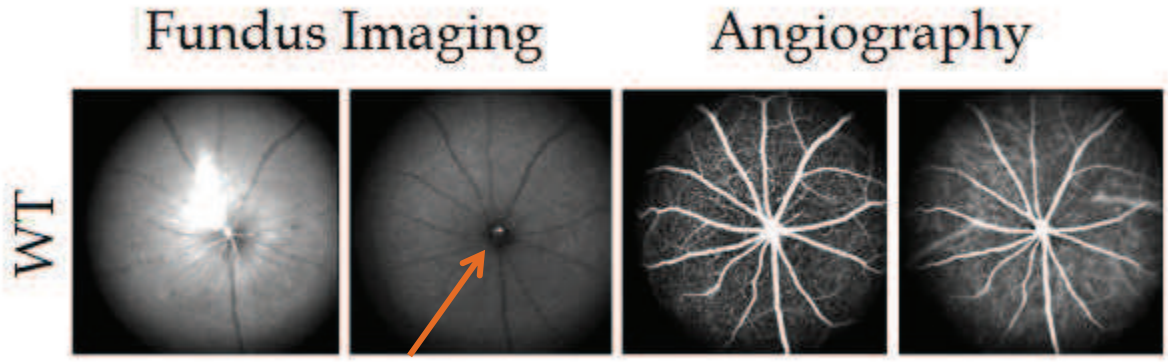


Figure 1 Using SLO one can visualize the morphology of the (inner) eye. This figure depicts examples of SLO images of a wildtype fundus (the image one sees when looking through the pupil in the eye using a SLO) (mouse). The optic nerve is present in the center of the eye and in the figure indicated by the arrow. The fundus of the rat is highly similar. The two images on the left depict the fundus using two different lasers. The two images on the right depict the blood vessels after visualization using a fluorescent dye (angiography). One uses this technique to find possible leakages.

SLO-OCT

The SLO module on our SLO-OCT combination is used to determine the general integrity of the retina. Large affected areas can be analysed with the use of this technique. In addition, the auto-fluorescence of the retina can be determined as well as the integrity of the blood vessels. To do this, different lasers are used (820 nm, 785 nm and 488 nm lasers). They shine through the pupil and illuminate the inside of the eye. The OCT module shows the morphology of the retina. All different layers can be distinguished and the thickness can be measured. Each measurement takes approximately 10 min (excluding (inhalation) anaesthesia).

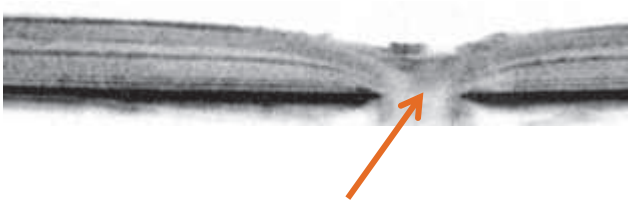


Figure 2 Example of OCT imaging of a WT retina (mouse). The optic nerve is indicated by the arrow. All the layers of the retina are visible when using this technique. The structure of the (WT) rat retina is highly similar.

ERG

By using non-invasive ERG measurements, the electric activity of the retina is determined. Once analysed, the data will show the activity of the rods (scotopic, a-wave, dark adapted animals), the cones (photopic, light adapted animals), the bipolar cells (scotopic, b-wave, dark adapted animals), the retinal pigment epithelium (RPE) and all other cell activities (oscillatory potentials) when stimulated with light. Each measurement takes approximately 25 minutes (excluding anaesthesia incubation). Two examples of ERG measurements in mice are presented in the figure below. Rat ERGs are higly similar.

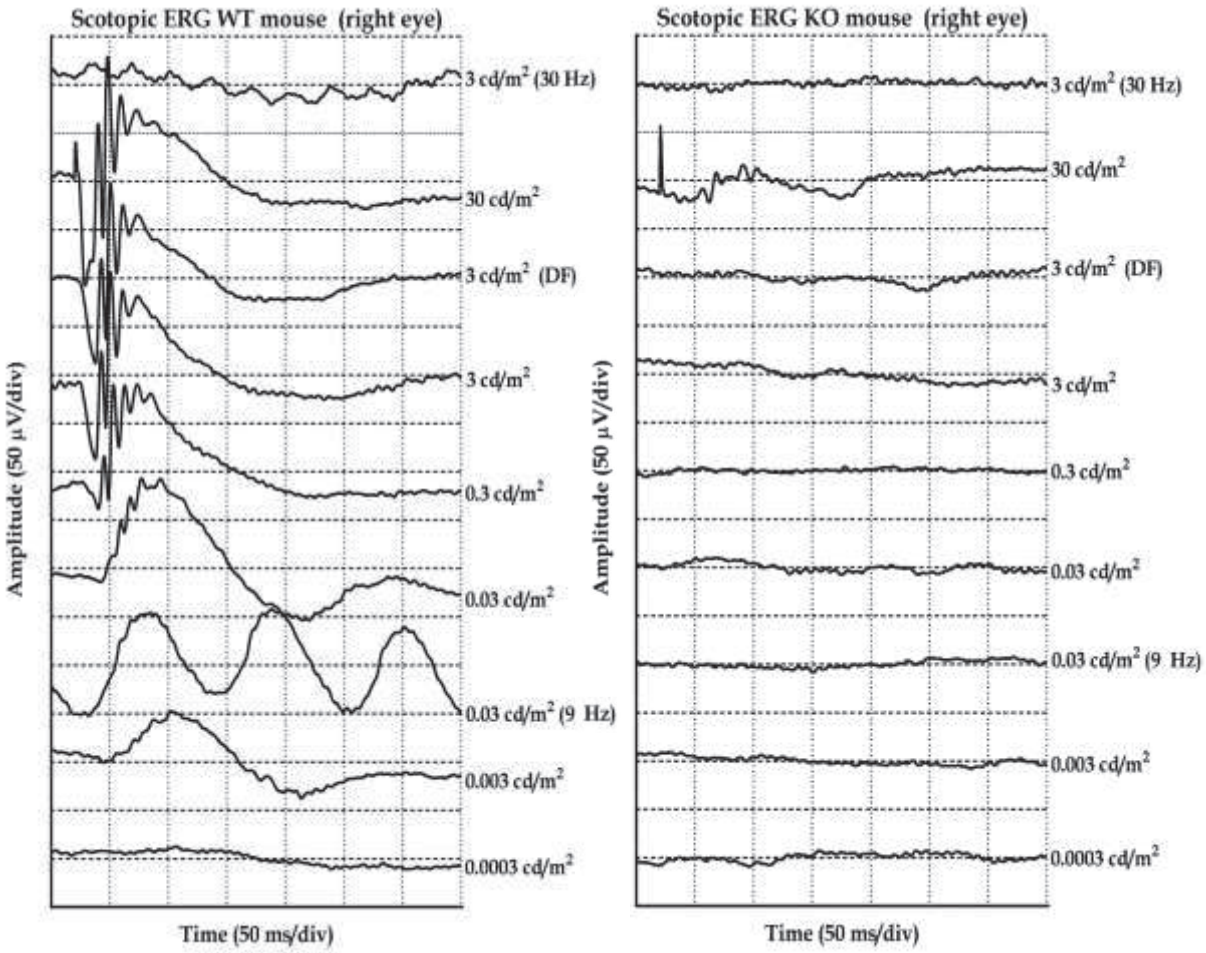


Figure 3 Examples of non-invasive ERG measurements performed on a WT mouse (left) and a [redacted] knockout (KO) mouse (right). The activity of the retina (its electric signals) are recorded in an electroretinogram (ERG). The activity is measures upon a response to a flash of light. Within this measurements several light intensities are used, going from 0.0003 cd/m2 (lowest) to 30 cd/m2. Furthermore, a flicker light is used (9 Hz and 30 Hz) and a double flash (DF). It is clear from this figure that the WT mouse has a retinal response to light, while the [redacted] KO mouse does not show any response. This makes sense since this KO mouse doesn't have an active visual cycle. A scotopic ERG measurement is performed in complete darkness, in this case only the rods are activated.

Pupillometry

The contraction of the pupil is a reflex as a result of exposure to light. This reflex is absent in animal models with a visual impairment. We will non-invasively measure the contraction of the pupil, with the use of infra-red video recordings, after exposure to light stimuli. This experiment is important to test the brain stem mediated feedback function and to see whether the signals are completely processed from the retina to the brain and back. Each measurement takes approximately 10 min.

Behavioral tests

We will also perform non-invasive behavioral tests which are based on vision. The behavior of the animals will be assessed using for example a light/dark room (see Figure 44). It is the nature of animals such as mice

and rats to hide from extensive light if they have the choice. In this experiment a cube is used which is half darkened. Animals which have normal vision will spend most of the time in the dark area, whereas animals which are blind will not notice the light and will therefore switch areas more and will be found more often in the bright area. The animal's movements will be recorded with video cameras and scored later. The importance of this experiment is to show whether the information from the retina to the visual cortex is correctly processed. Each test takes approximately 30 min. The tests can be performed under different light intensities in the bright compartment.

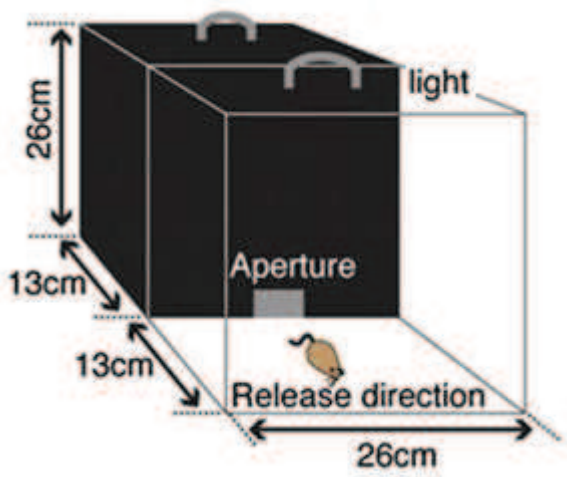


Figure 4 A light/dark chamber to test vision. These behavioral experiments are important to test whether information from retina is correctly processed to the visual cortex and other areas in the brain. Avoidance of the bright compartment will show us whether this is the case or not.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Breeding

The amount of animals which are kept in stock of the genetically modified lines, will be kept to the necessary minimum, i.e. usually one breeding trio per line and their most recent offspring. To ensure compatibility and prevent backcrossing, all animals will be maintained on a single genetic background [redacted] This will all be done by experienced personnel from the animal research centre.

Phenotyping

For the determination of the number of animals needed for [redacted] is according to literature and based on our previous experiences. In order to estimate the sufficient number of animals, we used the program G*power (http://www.gpower.hhu.de/), assuming a clear and consistent change (effect size = 0.8), a power of 0.8 and a significance level of 0.05. In addition, we will use the sample size calculator of the LASEC to determine sample size. Both methods will be combined. See the other appendices for the exact numbers of animals used in the specific experiments. [redacted]

See section B for the estimated numbers. The number of animals which need to be bred is based on the numbers described in appendix 3 (experimental animals).
A timeline of the experiments will be provided in all other appendices.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Breeding

Animals are (transgenic) [redacted] mice and rats. Both species need a [redacted], because the

[redacted] The animals which are used in the experiments have mutations in genes which are essential for normal vision. They will become blind over time. The use of transgenic models is essential because the diseases which are studied in these experiments have a genetic origin. Moreover, the disease in the models mimic the disease in humans. To test the efficacy of the new therapy, genetically blind animals are needed.

Animals of both sexes will be used in (early) adulthood. This appendix covers breeding of genetically modified animals with the possibility of offspring experiencing discomfort. This is the case for all heterozygous breeding of strains, which results in ~25% homozygous mutant offspring that is known to suffer low discomfort.
These crossings are crucial to this project, as they provide the strongest visually impaired phenotype, and thus, enable us to investigate whether a new therapeutic approach would recover the phenotype.

Origin

[redacted] This will be performed by a company which is specialized and very experienced in the generation of [redacted]
In addition to the [redacted] the mutant mice lines for [redacted] are already well-established. Heterozygotes for breeding will be taken from our own stock, and bred according to well-established standard protocols. Their homozygous mutant offspring (both sexes!) will be used for experiments. Heterozygote and wild-type animals will be either used for breeding and experiments or terminated.

Estimated numbers

[redacted]
For determining [redacted] From experience we know that in the two groups [redacted] we need 7 animals per group. This makes 28 ([redacted] x 2 groups x 7 animals per group) animals for both groups over [redacted]. Taking into account that some of the measurements are not usable (<10%) we would set the maximum amount of animals used for the [redacted] at 32. If we can get good and reliable results with less animals we will not use all animals. Both sexes can and will be used.

Experimental animals for other appendices
From previous experience, we estimate to need **maximally** 840 genetically modified rats and **maximally** 170 genetically modified mice over the next five years to perform the experiments described in this proposal. The numbers are based on calculations made in the appendix 3 (experimental animals).
Only the homozygotes are included in these numbers. Wild type animals from the same heterozygote-heterozygote crossings will be used as controls where needed to have breeding excess as low as possible. The numbers may vary between strains, depending on necessity of certain animals. However, the total number of genetically modified animals will not exceed the numbers described here. Both sexes can and will be used.

In summary: **for the next five years,** a maximum of 32 genetically modified rats will be used for phenotyping of the new rat model, a maximum of 840 genetically modified rats are used in appendix 3 and a maximum of 170 genetically modified mice are used in appendix 3.
Breeding without discomfort (not included in this appendix): 140 wildtype rats (appendix 2), 200 wildtype mice (appendix 4), 112 wildtype rats (appendix 3) and 112 wildtype mice (appendix 3) see the table below.
In the **very worst case scenario** that we have to use all animals that we have described within this project proposal (appendix 1-4), we will need a total of maximum 1156 rats and maximum 480 mice within the next five years.

Experiment	WT rat	[redacted] rat	WT mouse	[redacted] mouse
App. 1 phenotyping	32	32	-	-
App. 2	140	-	-	-
App. 3	112	840	112	168
App. 4	-	-	200	-
Total	284	872	312	168

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement

Immortalized cell lines and primary cultures are well-suited to research into the basic and molecular effects, but they do not reflect complete *in vivo* systems (in our case; the eye).
For our[redacted] it is essential to use animal models. One cannot re-create a functioning eye *in vitro* and follow its performance, especially not in the context of the whole animal. It is important to follow the effects over time in animal models to generate data about efficiency, efficacy and safety.

Reduction

For these experiments non-invasive screening and imaging techniques are used. This means that the animals which are used in the experiments can be followed and re-examined over time. It is therefore not necessary to use new animals for every time and measuring point. In addition, at the end of the experiments. Tissues will be isolated to answer any sub-goals and questions which are set on a molecular and biochemical level.
We will match[redacted], within each litter as much as possible, thus allowing us to have[redacted] Both sexes can and will be used.

Refinement

Before the proposed experiments will be started, all investigators have practiced extensively to master all the techniques which are used. Hereby, mistakes are limited as are unsuccessful measurements.
The screening techniques (SLO, OCT and ERG) have been improved and optimized before starting the experiments. Human endpoint have been set and pain treatments will be applied when necessary. Besides the application of the anaesthesia, measurements are non-invasive (except for the intervention itself). There will be no pain. The animals will have environmental enrichment and will be housed socially (where possible).

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

During breeding, all animals will be kept in a strictly isolated environment to minimise chances of contamination. Animals will be monitored at least three times a week, and any animal showing signs of any extensive distress will be euthanized according to the humane endpoints.
All contributors of this project are trained specifically to perform all measurements and interventions to minimise complications and animal suffering, pain or fear. Injection anaesthesia will be used with perioperative analgesia which also has pain killing effects 6 hours after the treatment while animal recovery

period. To prevent infection, antibiotics will be applied and/or immune suppressors.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This research is novel and has not yet been performed based on our knowledge of existing literature and in exchange with national and intrnational experts in the field.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X no individual housing No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

Breeding only No > Continue with question I.

[redacted] Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Anaesthesia including perioperative pain relief when applicable is used, which is according to our experience enough to prevent any pain during and after procedures.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

The animals which are maintained under this protocol are those where moderate discomfort is expected in 25% of the mutant offspring of heterozygous – heterozygous breeding that will be homozygous mutants (but no pain!). They will develop (partial) blindness. Bodyweight and other read-outs for animal welfare will not change. One cannot tell which animal is blind, they behave normally.

Explain why these effects may emerge.

The genes which are mutated are essential for normal vision. Once mutated, cellular and molecular processes are disturbed and blindness will be developed.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Animals with [redacted] are blind, but since animals such as mice and rats are not dependent on their vision, they do not show any change in behaviour (under normal housing circumstances). In the very rare case that a humane endpoint is reached, animals will be terminated.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

Regulation and guidelines according to FELASA are applied.
Despite the best countermeasures, animal might sometimes show signs of undue distress. Outward signs such as a ruffled fur coat or wounds and behavioural signs such as limping, hunched back or immobility will be taken as a sign for undue distress and the animal will be sedated an euthanized immediately.

Indicate the likely incidence.

This is not likely <1%.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Marking / tissue sampling for genotyping (mild, once): The animals will be marked by an appropriate method such as ear punching after the first postnatal week, which only causes momentary discomfort. The resulting samples will be used for genotyping. In rare instances, due to technical failures in genotyping a second sample may need to be taken.

Procedure	Discomfort level	%
(Partial) blindness as a result of a [redacted]	Moderate	100% [redacted]
Sacrificing	Mild	100% [redacted]
Anaesthesia for non-invasive screening	Moderate	100% [redacted]
Behavioural assay	Mild	100% [redacted]

The cumulative level of suffering will not exceed moderate in all animals.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

If the animals are not going to be used in other procedures, they will either be maintained in IVC for stock breeding [redacted]) or are sacrificed (for tissue harvesting). After the experiments (see other appendices), the animals will be terminated to harvest their eyes and blood for further analyses.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11400
1.2	Provide the name of the licenced establishment.	VUmc
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: [redacted] (setup of the method)

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Primary goal

The primary goal of these experiments is to develop the first steps for the generation of a new therapeutic intervention for patients with retinal degenerative diseases. For this, it is essential to test the efficacy and safety of the method. For this purpose, animal models (mice and rats) were specifically selected. The goal of the experiments described in this appendix is to set up [redacted] in our lab.

General design

This appendix covers the experiments in which rats are used to set up all handling procedures needed for the experiments in our lab. This is essential to train the persons involved in the experiments and to make the intervention as reproducible and effective as possible. F[redacted]

Wildtype animals will be taken from stock breeding. All procedures will be carried out in the building complex of the animal facility.

Initially, animals will be used for the handlings and terminated afterwards (group 2.1). This to minimize animal suffering due to unexperienced contributors (technical staff and new employees).

Once all handlings are familiar, we will use animals that will wake up after transplantation procedures and

follow them for a few weeks to see whether the handlings themselves have adverse effects (group 2.2).

We will subsequently proceed on the next group of animals with [redacted]; group 2.3) to test preliminary effectivity.

Once we are convinced that the animals are not suffering more than necessary, we will proceed to the next appendix (experimental animals). Read-outs are SLO-OCT and ERG measurements (described in appendix 1), eye morphology (*in vivo* and immunohistochemistry) and animal welfare.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

The experimental animals in this appendix are divided into three groups (2.1; Wildtype animals which are terminated, 2.2; Wildtype animals which will wake up and 2.3; Experimental [redacted]

Group 2.1: Animals will be anesthetized. Full anaesthesia will be confirmed by pinching the paw. All animals (also the other groups) will be kept warm during the entire procedure. Body temperature will be monitored during the whole procedure. The eyes of the animals will be anesthetized locally and dilated with eye drops. A [redacted] To maximize the use of each animal, both eyes will be used. The animals are not allowed to wake up again and will be terminated. Both sexes can and will be used. Animals are in the young-adolescent stage of their life.

Group 2.2: In case of scotopic (in the dark) ERG measurements, animals will be dark-adapted for at least 1 hour before the experiment and anesthetized. Full anaesthesia will be confirmed by pinching of the paw. The eyes of the animals will be locally anesthetized and dilated using eye drops. Scotopic and/or photopic (in the light) ERG measurements will be performed followed by SLO-OCT measurements (=baseline). [redacted] One eye will serve as a control (will [redacted] and the other eye will [redacted] The exact procedure is determined in group 2.1. After all procedures, correct [redacted] will be confirmed by using another SLO-OCT measurement. The animal is allowed to wake-up and antibiotic eye drops are applied. Follow-up ERG and SLO-OCT measurements will be performed after a few days (5-30) of recovery. Animals will be kept for a maximum of three months after the first procedure and no more than 5 measurements (4 follow-ups) will be performed per animal. Both sexes can and will be used. Animals are in the young-adolescent stage of their life.

Group 2.3: Before the procedures which are described for group 2.2 will be performed, the animals will be anesthetized. Full anaesthesia will be confirmed by pinching of the paw. The eyes of the animals will be anesthetized and dilated using eye drops. Scotopic and/or photopic ERG measurements will be performed followed by SLO-OCT measurements (=baseline). After this, the animals will receive an intra venous injection [redacted] The optimal [redacted] needs to be determined first (these animals will not undergo the additional proceedings) using [redacted] (based on literature). After a few days (6-20), the animals will undergo the same procedures as did group 2.2. The animals will be followed for a maximum time period of three months. Both sexes can and will be used. Animals are in the young-adolescent stage of their life.

Justifications

As stated, the main goal of the experiments in this appendix is to develop (new) [redacted] techniques in our lab. We believe that we have enough background knowledge, manpower, experience and instruments to be successful. The non-invasive screening techniques ERG and SLO-OCT are useful to determine success of [redacted]. Especially for animals which will wake up after procedures, these techniques are nice because they leave no extra damage to the animal. General anaesthesia, combined with extensive use of local anaesthesia and where needed post-operative analgesics, ensure that the animals suffer as little discomfort as possible during [redacted]. In addition, the strict post-operative surveillance regime we have planned will safeguard against undetected discomfort due to recovery.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

From the breeding stock, **both females and males** will be used, reducing breeding excess.
In order to estimate the animal numbers required for experiments we will use our previous knowledge and preliminary results from previous experiments where we also set up new methods.
For the determination of the number of animals needed [redacted] is according to literature and based on our previous experiences. In order to estimate the sufficient number of animals, we used the program G*power (http://www.gpower.hhu.de/), assuming a clear and consistent change (effect size = 0.8), a power of 0.8 and a significance level of 0.05. In addition, we will use the sample size calculator of the LASEC to determine sample size. Both methods will be combined.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

All experiments will be performed with wildtype [redacted] of both sexes. The rats will be taken from general breeding stock (breeding without discomfort). The rat model is an ideal model organism for this type of research, because it is a mammal and therefore phylogenetically closely related to humans. In comparison to mouse models, rats have a bigger eyes and it is therefore better possible to [redacted]. Rats share large genetic similarity with humans, and their eye morphology is roughly similar. Nevertheless, we will try to set up the transplantation procedure also in mice (Appendix 4) for the simple reason that multiple transgenic mouse models are already available for retinal degenerative diseases. And since these diseases are heterogeneous, it is also important to test the intervention in different genetic backgrounds. Taken together, these factors mean that at this point of our

research, rats are the model of choice for our experiments.

Estimated numbers
Since the experiments, which will be performed with the animals in this appendix, are based on a desirable success rate, the estimation that we can make here is rough and based on our previous experience. We expect to need approximately 140 animals (groups 2.1+2.2+2.3) as described below. Both sexes will be used.

Group 2.1: Animals will be terminated after procedures.
We will continue experiments when the success rate of our procedure exceeds approximately 60%. This is based on the success rate of our [redacted]. %. This 60% success rate is set based on our previous experience. The procedure of [redacted] These are known reasons based on prior experience which account for the 40% failure rate. These animals cannot be included in the follow-up measurements. If we find that this is the case, a human endpoint has been reached and the animals will be terminated. We are preparing technical improvements to hopefully be able to decrease this failure rate (see below) but currently calculated the number of required animals based on the currently used method.
A successful [redacted] is performed when [redacted] Little to no damage will be done to the hosting animal. [redacted] in which a cataract (touching of the lens, which blurs vision) or extensive intraocular bleedings are caused is not considered successful. To reach this we will use a maximum of 50 animals. This is estimated based on our previous experience in performing similar [redacted] in mice.

Group 2.2: Animals which are allowed to wake up after procedures and follow-up.
Once we are certain that we can perform [redacted] good enough to allow animals to wake up, we will follow at least 10 animals for a maximum time period of 1 month to determine any adverse effects caused by unexpected technical problems. Both eyes will be used for intervention within this group (either both [redacted]
Given the success rate of 60% and backup for non-statistically significant results, we will use a maximum of 30 animals. In total we will not exceed the total number of 30 animals within this (2.2) group.

Group 2.3: [redacted] to obtain preliminary data about effectiveness.
In this group wildtype animals will be used which, [redacted] Within these animals one eye will serve as a control. Given a success rate of [redacted] of 60% [redacted] and minimal groups of n=6 per group we would estimate the following numbers:
6 (n=6) x 2 [redacted] x 2 [redacted] = 24 (=60%) so 40 animals.
In addition, within this group animals will also be used to determine the [redacted] to use in rats. We previously did this in mice. We will use a [redacted] in duplicate), after this we narrow it down [redacted] (in triplicate) to determine the [redacted]. Our estimation will be [redacted] = 19 animals.
In total we will not exceed the total number of 60 animals within this group (2.3).

Life stages
Animals will be used at the (young) adult stage. We chose to use adult animals for the simple fact that their eyes are grown the biggest possible. In addition, we will use genetically modified rats after the [redacted]

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
These experiments will only start once we have sufficient knowledge from *ex vivo* experiments.
Immortalized cell lines and primary cultures are well-suited to research into the basic and molecular effects, but they do not form complete *in vivo* systems such as eyes. For these experiments it is essential to use animal models. One cannot re-create a functioning eye *in vitro* and follow its performance. It is important to follow the effects over time in animal models to generate data about efficiency, efficacy and safety.

Reduction
Before these experiments are started, all contributors have practiced extensively to master all the techniques which are used. Hereby, mistakes are limited as are unsuccessful measurements.
For these experiments non-invasive screening and imaging techniques are used. This means that the animals which are used in the experiments can be followed over time. It is therefore not necessary to use new animals for every time and measuring point. In addition, at the end of the experiments. Tissues will be isolated to answer any sub-goals and questions which are set.
We reduce variability in our experiments by using littermate controls wherever possible. This allows us to reach stronger effect sizes with smaller groups of animals due to lower variability. In addition, both males and females will be used, reducing excess of breeding. Technical refinements are in [redacted] to lower the percentage of drop-outs (see below).

Refinement
The screening techniques have been improved and optimized before starting the experiments. All testing which can be done ex-vivo will be done. Humane endpoints have been set and pain treatments will be applied when necessary. Besides the application of the anaesthesia, follow-up measurements are non-invasive. The animals will have environmental enrichment and will be housed socially (where possible). Technical refinements are in [redacted] thereby serving as refinedment that may directly lead to reduction as mentioned above.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be monitored at three times a week, and any animal showing signs of excessive distress will be processed according to the humane endpoints. [redacted], the animals will be kept under anaesthesia, sufficiently deep to feel no pain (periodically assessed via paw pinching reflex), but sufficiently light to still breathe autonomously. [redacted] will be carried out in aseptic conditions with [redacted], in specialized surgery rooms of the animal facility. We will administer antibiotics at the end [redacted] (eye drops) and eye salve. If necessary, we will introduce the administration of general analgesic at the [redacted] to reduce pain during recovery. The analgesic (and/or antibiotics) will be re-administered if the animal shows any sign of distress in the weeks [redacted].

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This research is novel and has not yet been performed based on our knowledge of existing literature and in exchange with national and international experts in the field.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Gas or injection anaesthesia will be used as well as peri-operative analgesia.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

[redacted]: Although the eye is immune-privileged, there is a low risk of an inflammatory reaction at the [redacted]. This will be closely monitored to ensure that the animal does not develop complications.

Explain why these effects may emerge.

[redacted] The risk of inflammation is low. This risk will also be minimised by [redacted], thorough training of any researcher and staff involved, standard application of antibiotic eye drops and close monitoring of the animal [redacted]

Indicate which measures will be adopted to prevent occurrence or minimise severity.

[redacted] will be performed by experienced and thoroughly trained scientists and/or technicians. This is crucial to minimize the risks during the procedures. Furthermore, the animals will be closely monitored during and after all procedures to ensure that they do not suffer undue distress.

[redacted] The animal will be kept warm (using a heating pad) [redacted] until coordinated movement is visible, then placed in its home cage and monitored until alert. If the animal shows signs of pain or distress from [redacted], it will be administered analgesics as needed. If any sign of an inflammatory reaction is seen, the animal will be given antibiotics and anti-inflammatory medication as needed. Following [redacted] the animals will be closely monitored.
In all cases, if the animal shows bodily or behavioural symptoms indicating undue distress, it will be euthanized according to the humane endpoints outlined below.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

Regulation and guidelines according to FELASA are applied.
Despite the best countermeasures, animal might sometimes show signs of undue distress. Signs such as a ruffled fur coat or wounds and behavioural signs such as limping, hunched back or immobility will be taken as a sign for undue distress and the animal will be sedated an euthanized immediately.
For [redacted], the animal will be monitored daily [redacted], and the [redacted], behaviour and level of activity will be watched. If the animal does show any signs of distress, it will be given analgesics and antibiotics. In case the animal does noy show improvement within 48 hours after treatment, it will be euthanized.
If animals cannot be used for follow-up measurements (40% drop-out), the animals will be euthanized. This will be, for example, when [redacted] Technical refinements are in [redacted] to lower this percentage in future experiments.

Indicate the likely incidence.

The likely incidence is very low in the case of side-effects originating from [redacted]. It is low for adverse [redacted] outcomes such as inflammation.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Procedure	Discomfort level	
Administration of [redacted]	Moderate	Group 2.3; 100%
Anaesthesia	Moderate	100%
[redacted]	Moderate	Group 2.2, 2.3; 100%
Follow-up measurements	Mild	100%

Administration of [redacted] (moderate, <1 minute, once): Group 2.3 will receive an i.v. injection containing [redacted] once.
Anaesthesia application for procedures (moderate, <2 minutes, max. 6 times): The animal will be sedated with an appropriate sedative such as isoflurane or a mixture of ketamine/xylazine and the absence of pain reception will be tested via paw pinching (absence of a reflex indicates total absence of pain sensation).
[redacted]: mild, [redacted], recovery: moderate, ca. 1 week; once): The [redacted] will be done under anaesthesia, and pain will be reduced post-operation by application of analgesics as necessary. Yet, during the recovery period, [redacted] will induce some moderate discomfort to the animal. Usually, the animals take a few days to recover, depending on the health status of the animal. After recovery, animals will generally not suffer further distress.
Follow-up measurements: non-invasive measurements will be performed under anaesthesia (see above).

The cumulative discomfort will not exceed moderate for animals within this appendix.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals will sacrificed at an appropriate time [redacted] for organ harvesting (usually eyes). Tissue will be used for further *ex vivo* analyses.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11400
1.2	Provide the name of the licenced establishment.	VUmc
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Experimental [redacted] in animal models for retinal degenerative diseases and its safety.

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Primary goal

The primary goal of these experiments is to develop the first experimental steps for the generation of a new therapeutic intervention for patients with retinal degenerative diseases. For this, it is essential to test the safety and efficacy of the methods in animal models. The goal of the experiments described in this appendix is to use the previously (appendix 2 and 4) developed [redacted]

General design

This appendix covers the experiments in which rats (and mice, **if** the appendix 4 pilot experiment has succeeded) will undergo [redacted] Animals are divided into six groups.

Group 3.1: [redacted]
Group 3.2:
Group 3.3:
Group 3.4:
Group 3.5: Safety in wildtype animals.
Group 3.6: [redacted]

The functional difference of [redacted] has not yet been shown. It is possible that there is a difference, or it is possible that there is no difference. It essential to know this, before we can make a step towards a human clinical trial.

We will use animals with a [redacted] induced blindness, including [redacted] In our studies we are aiming to be successful with our [redacted] in two rat models [redacted] and two mouse models [redacted] Our aim here is to perform the [redacted] successfully in rats and mice. If we can show that we are able to generate similar results in the [redacted] we can conclude that the [redacted]. This would open a tremendous window of genetic models which are available in mice only. Please note here that we will only proceed with these mouse experiments once we have succeeded to perform sufficiently in rats as described in appendix 4.

Table 1 An overview of chemically and/or genetically induced blindness in animal models.

Genotype (rat/mouse)	Manipulation/age	RPE status	Phenotype
Wild type	None	Normal	Normal
Wild type	[redacted]	Structure loss	[redacted]
Wild type	[redacted]	Structure and function loss	[redacted]
[redacted]	Young adolescence	Function loss	[redacted]
[redacted]	Young adolescence	Function loss	[redacted]
[redacted]	Older	Function loss	[redacted]
[redacted]	[redacted]	Structure and function loss	[redacted]

We will perform our previously setup [redacted] (appendix 2 and 4) only once we are convinced that we can perform very well. Read-outs are SLO-OCT and ERG measurements (described in appendix 1), behavioural experiments, eye morphology (*in vivo* and immunohistochemistry), expression profiling (after termination) and animal welfare.
We are aiming to answer the questions which we have set in the project proposal document.

Justification
The [redacted] is a previously used model in ophthalmology research. Our choice for rats over mice in the first experiments is a realistic one, since it has been shown that [redacted]
All animals will be followed for a maximum time period of a year. Within this window, we are convinced that any possible side-effects of the [redacted] will show. We expect also a functional recovery within this window.
The non-invasive techniques SLO, OCT and ERG will give as enough (functional) data to make any conclusions.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Wildtype animals and genetically modified animals will be taken from stock breeding (for breeding with discomfort, see appendix 1). All procedures will be carried out in the building complex of the animal facility. The experimental animals are divided into six groups. Animals will be used in (young) adulthood. Both sexes can and will be used.

Group 3.1: [redacted]
Within this group we will aim to use two models in rat and if possible two models in mouse. These will include [redacted] (group 3.1a) and [redacted] (group 3.1b).
Group 3.1b will undergo [redacted], group 3.1a will not.
Within this group we have several variables.
- Treated animals versus non-treated animals
[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Baseline ERG/SLO-OCT (and follow-up procedure)
In the case of scotopic ERG measurements, animals will be dark-adapted for at least 1 hour before the experiment. Injection and/or gas anaesthesia will be applied. Full anaesthesia will be confirmed by pinching of the paw. The eyes of the animals will be locally anesthetized and dilated using eye drops. Scotopic and/or photopic ERG measurements will be performed followed by SLO-OCT measurements. The animal will be kept warm during the whole procedure and body temperature will be monitored. In the case of an intervention, this will be performed within one anaesthesia. In the case of group 3.1b, animals will receive an intra venous injection with the [redacted] (appendix 2/4). The intervention will take place after a few days (5-20).

The intervention
Animals will be anesthetized and full anaesthesia will be confirmed by pinching the paw. All animals (also the other groups) will be kept warm during the entire procedure. Body temperature will be monitored during the whole procedure. The eyes of the animals will be anesthetized locally and dilated with eye drops.
[redacted] could be confirmed by using an additional SLO-OCT measurement. The animal is allowed to wake-up and antibiotic eye drops are applied. If found necessary, the animals will be administered with cyclosporine A or a comparable immune suppressor through their drinking water. Follow-up ERG and SLO-OCT measurements will be performed after a few days (6-20)
of recovery. Animals will be kept for a maximum of 1 year after the intervention. No more than 6 follow-up measurements will be performed.

Experimental group	Overall discomfort	Duration of experiment
Group 3.1, 3.2 and 3.4	Moderate	Maximally one year, no more than 6 follow-up measurements
Group 3.5	Moderate	Maximally one year, no more than 6 follow-up measurements
Group 3.6	Moderate	Maximally one year, no more than 6 follow-up measurements

Group 3.2, 3.3 and 3.4 have the same experimental setup as group 3.1. The only difference between the groups is

Group 3.5 Safety in wildtype animals
As stated, this group of animals will be used to determine the safety of the procedure in wildtype animals. The animals will undergo the same procedures as the other groups (baseline SLO-OCT/ERG measurements, intervention and follow-up measurements) and will be followed over the course of a maximum of 1 year. We will screen the animals in particular for an , whether their vision is stable and whether or not .

Group 3.6
Within this group (experiments planned at the end of the project), we are aiming to apply
The rationale behind this experiment is two-fold:

Second, we aim to use this procedure also for monogenic retinal degenerative diseases, that have a genetic causative component. . Based on results in the previous experiments, we will aim to For example; if we have cells from a

[redacted]
We are aiming to be successfully apply this strategy in two animal models (rat **or** mouse, two genetic backgrounds). The exact genetic backgrounds are dependent on the patient cohorts which are available and on the previous experiments. In addition, depending on the outcomes of appendix (mouse pilot) and previous experiments, we will decide on whether to use mice or rats for these experiments. Last, we will decide [redacted] based on the previous experiments.

Genetic background 1 rat **or** mouse	Genetic background 2 rat **or** mouse
[redacted]	[redacted]
[redacted]	[redacted]

Justifications
As stated, the main goal of the experiments in this appendix is to set the first steps for the generation of a new therapeutic intervention for patients with retinal degenerative diseases. We believe that (once succeeded appendix 2 and 4) we have enough background knowledge, experience and instruments to be successful. The non-invasive screening techniques ERG and SLO-OCT are useful to determine success and generate *in vivo* data. These techniques will not leave extra damage to the animal. [redacted]
General anaesthesia, combined with extensive use of local anaesthesia and where needed post-operative analgesics, ensure that the animals suffer as little discomfort as possible [redacted] and recovery. In addition, the strict [redacted] we have planned will safeguard against undetected discomfort due to recovery.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The amount of animals which are kept in stock of the genetically modified lines, will be kept to the necessary minimum, i.e. usually one breeding trio per line and their most recent offspring. To ensure compatibility and prevent backcrossing, all animals will be maintained on a single genetic background [redacted] To reduce breeding excess, both males and females will be used.

For the determination of the amount of animals needed for [redacted] is according to literature and based on our previous experiences. In order to estimate the sufficient number of animals, we used the program G*power (http://www.gpower.hhu.de/), assuming a clear and consistent change (effect size = 0.8), a power of 0.8 and a significance level of 0.05. In addition, we will use the sample size calculator of the LASEC to determine sample size. Both methods will be combined. See the other appendices for the exact numbers of animals used in the specific experiments.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

All experiments will be performed with animals in a [redacted]
The wildtype animals will be taken from general breeding stock (breeding without discomfort). The genetically altered animals will be taken from (our) general breeding stock (breeding with discomfort, see appendix 1). Mice and rats are ideal animal models for this type of research, since it is a mammal and therefore phylogenetically closely related to humans. Rats are relatively easy genetically modified in comparison to larger-eyed animals such as pigs and rabbits. In comparison to rats, mice are even more easy to genetically modify. However, in comparison to mice, rats have bigger eyes and are therefore a better possibility for the [redacted]. If succeed in appendix 4 (mouse pilot) mice are also a possible animal model for [redacted]. In this case, mice have our preference, since a lot of genetically altered mouse strains are already available or easily can be generated. Since the diseases which we study are mostly heterogenetic, it is important to test our intervention in different genetic backgrounds.
Taken together, these factors mean that at this point of our research, rats and possibly mice are the model of choice for our experiments.

Estimated numbers
At this point in time, we have succeeded setting up [redacted] in rats and possibly in mice. If not, we will not proceed to this appendix and use the animals which are assigned to the experimental groups in this appendix.
We have assigned animals within this appendix to different groups as stated also above:
Group 3.1: [redacted]
Group 3.2:
Group 3.3:
Group 3.4:
Group 3.5: Safety in wildtype animals.
Group 3.6: [redacted].

Groups 3.1 t/m 3.4 can be generally summarized as follows:
Rats
2 models [redacted] x 4 conditions [redacted] x 2 [redacted] x 2 [redacted] = 2 x 2 x 4 x 2 x 2 = 56 "groups".
We expect to need 7 animals per group to reach statistical significance which means 56 x 7 = 392 animals. Since we aim in the setup of [redacted] of at least 60% and we expect an [redacted] drop-out of 10%, we expect to need (392 = 50%) a maximum 784 animals (rats) for the coming **five years**. This is however, the worst case scenario. We will only use the necessary amount of animals.

This 60% success rate is set based on our previous experience. The procedure of [redacted] These are known reasons based on prior experience which account for the 40% failure rate. These animals cannot be included in the follow-up measurements. If we find that this is the case, a human endpoint has been reached and the animals will be terminated. We are preparing technical improvements to hopefully be able to decrease this failure rate (see below) but currently calculated the number of required animals based on the currently used method.

Mice
In the case that we proceed with mice at this point (**depending on the outcome of appendix 4**) we will test 2 models [redacted] x 4 conditions [redacted] x 1 [redacted] = 2 x 4 x 1 x 1 = 8 "groups".
We expect in the case of mouse to need 7 animals per group to reach statistical significance which means 8 x 7 = 56 animals. Since we aim in the setup of the transplantation procedure for a realistic success rate of at least 60% and we expect a maximum drop-out [redacted] of 10%, we expect to need (56 = 50%) a maximum of 112 animals (mice) for the coming **five years**. This is, however, the worst case scenario. We will only use the necessary amount of animals.

Group 3.5: Safety in wildtype animals.
These experiments will be performed initially in rats, and if necessary also in mice.
We will test the safety of our procedure [redacted] in wildtype animals. This group can be summarized as follows: 1 animal model x 4 conditions [redacted] x 1 [redacted] x 2 [redacted] = 1 x 4 x 1 x 2 = 8 "groups". We expect to need 7 animals per group to reach statistical significance which means 8 x 7 = 56 animals. Since we aim in the setup [redacted] for a realistic success rate of at least 60% and expect a maximum drop-out [redacted] of 10%, we expect to need (56 = 50%) a maximum of 112 animals (rats and/or mice). This is however, the worst case scenario. We will only use the necessary amount of animals.

Group 3.6 [redacted]
These experiments will be preferably performed in mice, since multiple genetically mutated strains are already available and the new methodology can be applied to strains with other mutations. If we not

succeed in appendix 4, we will use rats in this group as an alternative.
This group can be summarized as follows: 2 genetic backgrounds x 2 conditions [redacted] = 2 x 2 = 4 "groups".
We expect to need 7 animals per group to reach statistical significance which means 4 x 7 = 28 animals (mice or rats). Since we aim in the setup of [redacted] for a realistic success rate of at least 60% and we expect a maximum drop-out [redacted] of 10%, we expect to need (28 = 50%) a maximum of 56 animals. This is, however, the worst case scenario. We will only use the necessary amount of animals.

To summarize the **maximum** amount of animals which will be used within this appendix (**within 5 years**):
Rats: 784 + 112 + **possibly** 56= maximally 952 (from which 840 genetically modified and 112 wild-type).
Mice: **possibly** 112 + **possibly** 112 + **possibly** 56 = maximally 280 (from which 168 genetically modified and 112 wild-type).

Life stages
Animals will be used at the adolescent – adult stage. We will use genetically modified rats/mice. These animals are born [redacted] Both sexes can and will be used.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
We have previously gained as much as possible knowledge from *ex vivo* experiments, including cell culture experiments and the usage of donor eyes (human). Immortalized cell lines and primary cultures are well-suited to research into the basic and molecular effects, but they do not form complete *in vivo* systems such as eyes. For these experiments it is essential to use animal models. One cannot re-create a functioning eye *in vitro* and follow its performance. It is important to follow the effects over time in animal models to generate data about efficiency, efficacy and safety.

Reduction
Before these experiments are started, all contributors have practiced extensively to master all the techniques which are used. Hereby, mistakes are limited as are unsuccessful measurements. In addition, at this point in time we have performed all procedures in appendices 2 (rats) and 4 (mice) and we are convinced that we can perform these very well.
For all experiments, non-invasive screening and imaging techniques are used. This means that the animals which are used in the experiments can be followed over time. It is therefore **not** necessary to use new animals for every time and measuring point. In addition, at the end of the experiments, the animals will not go to waste. Tissues will be isolated to answer any sub-goals and questions which are set.
We reduce variability in our experiments by using littermate controls wherever possible. This allows us to reach stronger effect sizes with smaller groups of animals due to lower variability. In addition, both males and females will be used, reducing excess of breeding. Technical refinements are in [redacted] to lower the percentage of drop-outs (see below).

Refinement
To guarantee the best possible environment and minimize chances of contamination for the animals during breeding, they will be housed in sterilized individually ventilated cages, receive sterilized food and drinking water, and only be handled under a flow hood.
The screening techniques [redacted] have been improved and optimized before starting the experiments. All testing which can be done ex-vivo has been done. Humane endpoints have been set and pain treatments will be applied when necessary. Besides the application of the anaesthesia, follow-up measurements are non-invasive. The animals will have environmental enrichment and will be housed socially (where possible). Technical refinements are [redacted] to lower the extend of damage and increase [redacted] thereby serving as refinedment that may directly lead to reduction as mentioned above.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be monitored at least weekly, and any animal showing signs of excessive distress will be processed according to the humane endpoints. [redacted], the animals will be kept under anaesthesia, sufficiently deep to feel no pain (periodically assessed via paw pinching reflex), but sufficiently light to still breathe autonomously. [redacted] will be carried out in aseptic conditions with sterilized tools, in specialized facilities. We will administer antibiotics [redacted] (eye drops) and eye salve. If necessary, we will introduce the administration of general analgesic [redacted] to reduce pain during recovery. The analgesic (and/or antibiotics) will be re-administered if the animal shows any sign of distress in the weeks [redacted]

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This research is novel and has not yet been performed based on our knowledge of existing literature and in exchange with national and intrnational experts in the field.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Gas or injection anaesthesia will be used as well as peri-operative analgesia.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

[redacted] Although the eye is immune-privileged, there is a low risk of an inflammatory reaction [redacted]. This will be closely monitored to ensure that the animal does not develop complications.

Explain why these effects may emerge.

[redacted]. The risk of inflammation is low. This risk will also be minimised by [redacted], thorough training of any researcher and staff involved, standard application of antibiotic eye drops and close monitoring of the animal [redacted]

Indicate which measures will be adopted to prevent occurrence or minimise severity.

[redacted] will be performed by experienced and thoroughly trained scientists and/or technicians. This is crucial to minimize the risks during the procedures. Furthermore, the animals will be closely monitored during and after all procedures to ensure that they do not suffer undue distress.
[redacted]: The animal will be kept warm (using a heating pad) [redacted] until coordinated movement is visible, then placed in its home cage and monitored until alert. If the animal shows signs of pain or distress [redacted], it will be administered analgesics as needed. If any sign of an inflammatory reaction is seen, the animal will be given antibiotics and anti-inflammatory medication as needed. [redacted] the animals will be closely monitored.
In all cases, if the animal shows bodily or behavioural symptoms indicating undue distress, it will be euthanized according to the humane endpoints outlined below.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

Regulation and guidelines according to FELASA are applied.
Despite the best countermeasures, animal might sometimes show signs of undue distress. Signs such as a ruffled fur coat or wounds and behavioural signs such as limping, hunched back or immobility

will be taken as a sign for undue distress and the animal will be sedated an euthanized immediately.
[redacted], the animal will be monitored daily[redacted], and the state [redacted], behaviour and level of activity will be watched. If the animal does show any signs of distress, it will be given analgesics and antibiotics. In case the animal does noy show improvement within 48 hours after treatment, it will be euthanized.

If animals cannot be used for follow-up measurements (40% drop-out), the animals will be euthanized. This will be, for example, [redacted] Technical refinements are in [redacted] to lower this percentage in future experiments.

Indicate the likely incidence.

The likely incidence is very low in the case of side-effects originating from needle trauma. It is low for adverse post-surgery outcomes such as inflammation.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Procedure	**Discomfort level**	
[redacted]	Moderate	Group 3.1 tm 3.4; 100%
Anaesthesia	Moderate	100%
[redacted]	Moderate	100%
Follow-up measurements	Mild	100%

Administration [redacted] (moderate, <1 minute, once): Group 2.3 will receive an i.v. injection containing [redacted]
Anaesthesia application for procedures (moderate, <2 minutes, max. 6 times): The animal will be sedated with an appropriate sedative such as isoflurane or a mixture of ketamine/xylazine and the absence of pain reception will be tested via paw pinching (absence of a reflex indicates total absence of pain sensation).
[redacted] recovery: moderate, ca 1 week; once): The [redacted] will be done under anaesthesia, and pain will be reduced [redacted] by application of analgesics as necessary. Yet, during the recovery period, [redacted] will induce some moderate discomfort to the animal. Usually, the animals take a few days to recover, depending on the health status of the animal. After recovery, animals will generally not suffer further distress.
Follow-up measurements: non-invasive measurements will be performed under anaesthesia (see above). The animals will be in the experiments for the maximum period of a year.
The cumulative discomfort will not exceed moderate for animals within this appendix.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals will sacrificed at an appropriate time after transplantation for organ harvesting (usually eyes). Tissue will be used for further *ex vivo* analyses.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Centrale Commissie Dierproeven

7.

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	11400
1.2	Provide the name of the licenced establishment.	VUmc
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 4 Type of animal procedure: PILOT: [redacted] in mouse (setup the method)

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Primary goal

The primary goal of these experiments is to set the first steps for the generation of a new therapeutic intervention for patients with retinal degenerative diseases. For this, it is essential to test the efficacy and safety of the method in animal models. The goal of the pilot-experiments described in this appendix is to set-up [redacted] in our lab.

General design

This appendix covers the experiments in which mice are used to set-up all experimental procedures needed for the pilot-experiments. This pilot is essential to perform because the most ideal animal model for retinal degenerative diseases is an animal model for which multiple transgenic lines are available. This is reasoned from the fact that retinal degenerative diseases are mostly genetically heterogeneous. For different patient groups, a different model is required. Up to now, the mouse is the only animal model in which multiple transgenic lines exist or can be easily obtained. However, the mouse eye is relatively small [redacted] We think that we can be successful in mice, but this requires a lot of experience (which we obtain during all rat experiments).

Furthermore, it is essential to train the contributors which are involved in the experiments as good as possible. For this pilot, either [redacted]. Within this pilot, we clearly incorporated checkpoints at which we reason whether or not to continue with the experiments.

Initially, animals will be used for the experimental procedures and terminated afterwards (group 4.1). This is incorporated to minimize animal suffering due to the performance of new proceedings. The main goal after this first step is [redacted] a new technique, we have to perform a pilot experiment first. Please note here that we will only continue to the next step once we are fully convinced that the animal will not suffer more than it should after waking up from the procedure.

The next group of animals will therefore consist of animals which are allowed to wake up after the procedure (group 4.2). We will follow the animals for a few weeks to see whether the experimental procedures themselves have any adverse effects. Please note here that we will only continue to the next step once we are fully convinced of our abilities to apply an intervention to [redacted] animals. If we notice during or after the procedure that the animals suffer more than they should (see humane endpoints), we will terminate them preterm according to the described methods.

Group 4.3 consists of animals which are [redacted]. This group is used to test preliminary effectivity of the intervention. Once we are convinced that the animals are not suffering more than necessary, we will allow the mouse to be an animal model within our studies and we will then proceed (include the mouse) to the experimental animals (appendix 3).

Read-outs within this appendix are non-invasive SLO-OCT and ERG measurements (follow-up, described in appendix 1), eye morphology (*in vivo* and *ex vivo* immunohistochemistry) and animal welfare.

Justification
As stated before, the mouse is the most ideal animal model available when one focuses on the availability of several transgenic lines. We will perform this pilot because [redacted] However, we think that this problem can be overcome. Especially once we have great experience using the same technology in rat eyes. Despite the size of the mouse eye, we are still aiming for availability [redacted]

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

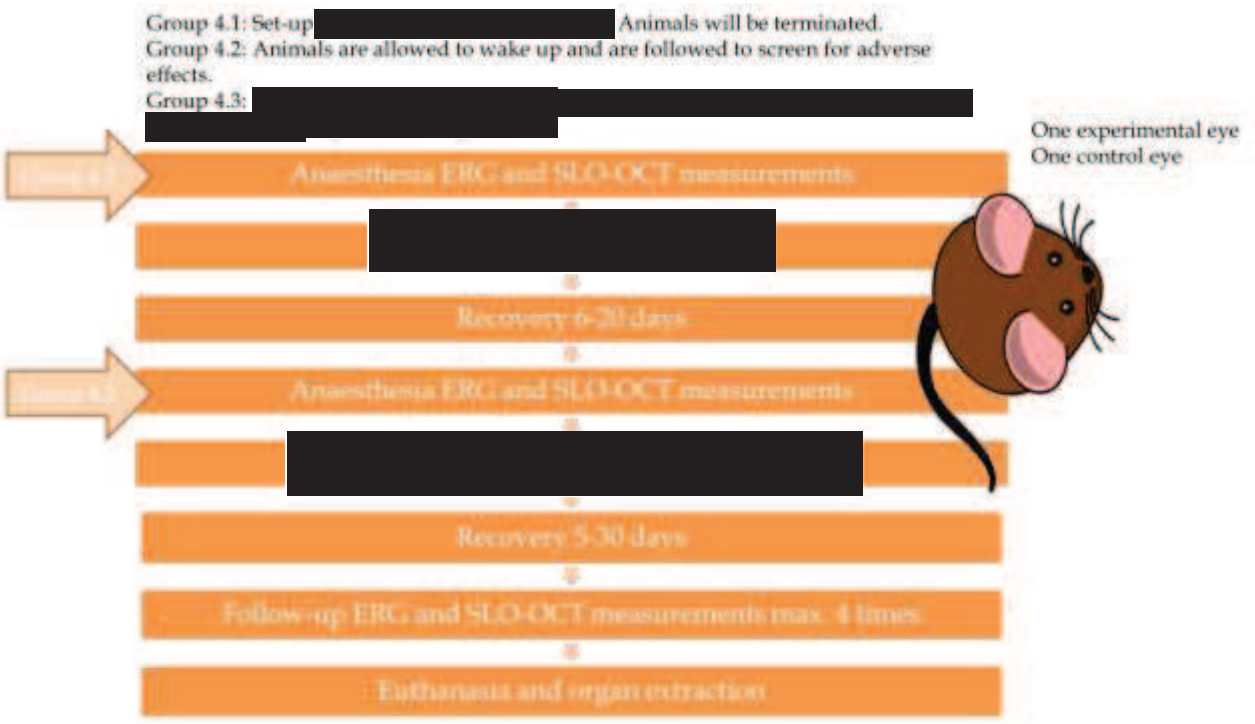
Wildtype animals will be taken from stock breeding. All procedures will be carries out in the building complex of the animal facility (VU-UPC, Amsterdam).
The experimental animals within this pilot are divided into three groups (4.1; Wildtype animals termination, 4.2; wildtype animals which wake up and 4.3; [redacted]).

Group 4.1: Animals will be anesthetized and full anaesthesia will be confirmed by pinching of the paw. All animals (also the animals in the other groups) will be kept warm during the entire procedure. Body temperature will be monitored during the whole procedure. The eyes will be locally anesthetized and dilated with eye drops. Using [redacted] To maximize the use of each animal (and minimize total animal numbers), both eyes will be used. To minimize animal suffering, the animals are not allowed to wake up again and will be terminated immediately after the intervention. Only when we are convinced that we can perform all procedures very well, we will allow the animals to wake up after procedures (= checkpoint).

Group 4.2: Since we want to follow the (wildtype) animals from this group after the intervention, we will perform baseline measurements by SLO-OCT and ERG. In case of scotopic ERG measurements, animals will be dark-adapted for at least 1 hour before the experiment and anesthetized. Full anaesthesia will be

confirmed by pinching of the paw. The eyes of the animals will be locally anesthetized and dilated using eye drops. Scotopic and/or photopic ERG measurements will be performed followed by SLO-OCT measurements (= baseline). [redacted] according to the methods setup in group 4.1. Within this group (4.2), one eye will serve as a control (will [redacted] and the other eye will [redacted] After all experimental procedures, [redacted] The animal is allowed to wake up and antibiotic eye drops are applied. If necessary, the animals will be administered with cyclosporine A or a comparable immune suppressor through their drinking water. Follow-up ERG and SLO-OCT measurements will be performed after a few days (5-30) of recovery. Animals will be kept for a maximum of 1 month after the first procedure and no more than 5 measurements (4 follow-ups) will be performed per animal. Once we are convinced that within the monitoring period of 1 month the animals do not suffer more than necessary and we are successful [redacted] we will continue with the next group of animals.

Group 4.3: The animals within this group will be used to determine preliminary effectivity within a [redacted] The animals will undergo the same procedures described for group 4.2. In addition to this, they will be anesthetized. Full anaesthesia will be confirmed by pinching of the paw. The eyes of the animals will be anaesthetized locally and dilated using eye drops. Scotopic (night vision) and/or photopic (day vision) ERG measurements will be performed followed by SLO-OCT measurements (=baseline). After this the animals will receive an intra venous injection [redacted] After a recovery period (6-20 days) the animals will undergo the same procedures as the animals in group 4.2.



Justifications

As stated, the main goal of the experiments in this appendix is to get the (new) [redacted] up and running in our lab using the mouse as animal model. We believe that we have enough background knowledge, experience and instruments to have a chance to be successful. After and during this pilot, we clearly decide whether we will continue with the mouse as an animal model, since we are not sure whether we will succeed due to the size of the mouse eye. The non-invasive screening techniques ERG and SLO-OCT are useful to determine success of the technique. Especially for the animals which are allowed to wake up

after the procedures, these techniques are beneficial, because they leave no extra damage to the animal. General anaesthesia, combined with extensive use of local anaesthesia and where needed [redacted] analgesics, ensure that the animals suffer as little discomfort as possible [redacted] and recovery. In addition, the strict [redacted] surveillance regime that we have planned will safeguard against undetected discomfort during recovery due to the procedures.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

From the breeding stock, both females and males will be used which reduces the excess of breeding. In order to estimate the animal numbers required for the pilot, we will consult our previous knowledge and experience from previous experiments. Within these experiments we also successfully set up new methods.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

All experiments within this pilot will be performed with wildtype [redacted] mice. The mice will be taken from the general breeding stock (breeding without discomfort). The mouse model is an ideal animal model for this type of research, because it is a mammal and therefore phylogenetically closely related to humans. The mice is easily genetically modified in comparison to all other animal models. This holds the main advantage of this animal model, since retinal degenerative diseases are mostly genetically heterogeneous. Mice share large genetic similarity with humans and their eye morphology is roughly similar. Taken together, it would be a great benefit if we are able to perform [redacted] in mice.

Estimated numbers
Since the experiments, within this pilot, are based on a screening of possibilities and are based on a desirable success rate, the estimation that we can make here is rough and based on our previous experience. We expect to need approximately 200 animals as described below. We will not exceed this amount of animals within this pilot.

Group 4.1: Animals will be terminated after procedures. These animals will be used to screen for possible strategies to perform [redacted]. We will continue experiments when the success rate of the selected procedure(s) exceeds 60%. This 60% success rate is set based on our previous experience. The procedure of [redacted] These are known reasons based on prior experience which account for the 40% failure rate. These animals cannot be included in the follow-up measurements. If we find that this is the case, a human endpoint has been reached and the animals will be terminated. We are preparing technical improvements to hopefully be able to decrease this failure rate (see below) but currently calculated the number of required animals based on the currently used method. To reach this, we will use a maximum of 100 animals.

Group 4.2: The animals are allowed to wake up after the procedure (if successfully performed) and will be followed over time. We will only allow the animals to wake up once we are certain that we are able to perform [redacted] sufficiently to limit animal suffering. We will follow at least 10 animals which received a [redacted] for a maximum time period of 1 month to determine any adverse effects due to technical problems. Both eyes will be used for intervention within this group [redacted] Given the success rate of 60% and backup for non-statistically significant results, we will use a maximum of 50. In total we will not exceed the total number of 50 animals within this group. We will use as little animals as possible.

Group 4.3: The animals within this group are firstly [redacted] functioning. We will only continue using the animals within this group once we are convinced that within the time period of follow up there are no unnecessary adverse effects resulting from the intervention (determined using group 4.2). We will have two animal models within this group: [redacted] Within these animals, one eye will serve as a control. Given a success rate of the [redacted] of 60% [redacted] and minimal group numbers of n=6, we would estimate the following numbers:

6 (animals per group) x 2 (models) x 2 [redacted] = 24 (=60%) so 40 animals.
We expect a drop-out percentage of 20% (based on our previous experience) during follow-up which makes a total number of 50 animals. We will use as little animals as possible.

Life stages
Animals will be used at the adult stage. We chose to use adult animals for the simple fact that their eyes are grown the biggest as possible. In addition, we plan to use genetically modified mice if we succeed setting up the [redacted]. These animals are born [redacted]

C. Re-use

Will the animals be re-used?

X No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
These experiments will only start once we have sufficient knowledge from *ex vivo* experiments and experience from previous experiments using the same animal model. Immortalized cell lines and primary cultures are well-suited to research into the basic and molecular effects, but they do not form complete *in vivo* systems such as eyes. For the experiments described here, it is essential to use animal models. One cannot re-create a functioning eye *in vitro* and follow its performance (yet). It is important to follow the effects over time in animal models to generate data about efficiency, efficacy and safety.

Reduction
For these experiments, non-invasive screening and imaging techniques are used. This means that the animals which are used in the experiments can be followed over time. It is therefore not necessary to use new animals for every time and measuring point. In addition, at the end of the experiments, the animals will not go to waste. Tissues will be isolated to answer any sub-goals and questions which are set. We reduce variability in our experiments by using littermate controls wherever possible. This allows us to reach stronger effect sizes with smaller groups of animals due to lower variability. In addition, both males and females will be used, reducing excess of breeding. By building in clear check-point between all steps of the experiment, we can decide whether we should proceed with the next group of animals. We will therefore not necessary use all animals which are described in this appendix. Technical refinements are [redacted] to lower the percentage of drop-outs (see below).

Refinement
Before the pilot will start, all contributors have been trained extensively to master all the techniques which are used. Hereby, mistakes are limited as are unsuccessful measurements.
All screening techniques have been improved and optimized before starting the experiments. All testing which can be done *ex vivo* will not require any animals. Humane endpoints have been set and pain treatments will be applied when necessary. Besides the application of anaesthesia, follow-up measurements are non-invasive. The animals will have environmental enrichment and will be housed socially (where possible). Technical refinements are [redacted] to lower the extend of damage and increase the [redacted] success thereby serving as refinedment that may directly lead to reduction

as mentioned above.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be monitored at least weekly and any animal showing signs of excessive distress will be processed according to the humane endpoints. [redacted], the animals will be kept under anaesthesia, sufficiently deep to feel no pain (periodically assessed via paw pinching reflex), but sufficiently light to still breathe autonomously. [redacted] will be carried out in aseptic conditions with sterilized tools in specialized facilities. We will administer antibiotics at the [redacted] (eye drops) and eye salve. We will use [redacted] analgesia and if necessary the analgesics will be repeated during recovery. The analgesic (and/or antibiotics) will be re-administered if the animal shows any sign of distress in the weeks [redacted]
If we doubt the successfulness of a [redacted], we will not allow the animal to wake up under any circumstances.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This research is novel and has not yet been performed based on our knowledge of existing literature and in exchange with national and intrnational experts in the field.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

X Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Gas or injection anaesthesia will be used as well as [redacted] analgesia.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

[redacted]: There is a low risk of an inflammatory reaction [redacted] in the eye. This will be closely monitored to ensure that the animal does not develop complications.

Explain why these effects may emerge.

[redacted]: Since [redacted]. The risk of inflammation is low. The risk will also be minimised by [redacted], thorough training of any researcher and staff involved, standard application of antibiotic eye drops and close monitoring of the animal [redacted]. When we are doubting the success [redacted] which results in a higher risk for animal suffering, we will not allow the animal to wake up.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

[redacted] will be performed by experienced an thoroughly trained scientists and/or technicians. This is crucial to minimize the risks during the procedures. Furthermore, the animals will be closely monitored during and after all procedures to ensure that they do not suffer undue distress. [redacted] The animal will be kept warm (using a heating pad)[redacted] until coordinated movement is visible, then placed in its home cage and monitored until alert. If the animal shows signs of pain or distress from [redacted], it will be administered analgesics as needed. If any sign of an inflammatory reaction is seen, the animal will be given antibiotics and/or anti-inflammatory medication as needed. [redacted], the animals will be closely monitored. In all cases, if the animal shows bodily or behavioural symptoms indicating undue distress, it will be euthanized according to the humane endpoints outlined below.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

X Yes > Describe the criteria that will be used to identify the humane endpoints.

Regulation and guidelines according to FELASA are applied.
Despite the best countermeasures, animal might sometimes show signs of undue distress. Outward signs such as a ruffled fur coat or wounds and behavioural signs such as limping, hunched back or immobility will be taken as a sign for undue distress and the animal will be sedated an euthanized immediately. [redacted], the animal will be monitored [redacted], and the state of [redacted], behaviour and level of activity will be watched. If the animal does show any signs of distress, it will be given analgesics and antibiotics. In case the animal does noy show improvement within 48 hours after treatment, it will be euthanized.

If animals cannot be used for follow-up measurements (40% drop-out), the animals will be euthanized. This will be, for example, when [redacted] Technical refinements are in preparation [redacted] to lower this percentage in future experiments.

Indicate the likely incidence.

The likely incidence is very low in the case of side-effects originating from needle trauma. It is low for adverse [redacted] such as inflammation.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Procedure	Discomfort level	
[redacted]	Moderate	Group 3.3: 100%
Anaesthesia	Moderate	100%
[redacted]	Moderate	Group 3.2 and 3.3: 100%
Follow-up measurements	Mild	Group 3.2 and 3.3: 100%

Administration [redacted] (moderate, <1 minute, once): Group 4.3 will receive an i.v. injection containing [redacted] once.
Anaesthesia application for procedures (moderate, <2 minutes, max. 6 times): The animal will be sedated with an appropriate sedative such as isoflurane or a mixture of ketamine/xylazine and the absence of pain reception will be tested via paw pinching (absence of a reflex indicates total absence of pain sensation).
[redacted], recovery: moderate, ca 1 week; once): The [redacted] will be done under anaesthesia, and pain will be reduced post-operation by application of analgesics as necessary. Yet, during the recovery period, [redacted] will induce some moderate discomfort to the animal. Usually, the animals take a few days to recover, depending on the health status of the animal. After recovery, animals will generally not suffer further distress.
Follow-up measurements: non-invasive measurements will be performed under anaesthesia (see above).
The cumulative discomfort will not exceed moderate for animals within this appendix.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals will sacrificed at an appropriate time [redacted] for organ harvesting (usually eyes). Tissue will be used for further *ex vivo* analyses.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes

Format DEC-advies

A. Algemene gegevens over de procedure

1. Aanvraagnummer:
Het NVWA nummer is 11400

2. Titel van het project:
Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration

3. Titel van de NTS:
De experimentele therapeutische transplantatie van stamcel-retinaal pigment epitheel in diermodellen voor retinale degeneratie

4. Type aanvraag:
Nieuwe aanvraag projectvergunning

5. Contactgegevens DEC:
   - naam DEC: *Vrije Universiteit Amsterdam / VU medisch centrum*
   - telefoonnummer contactpersoon: [redacted]
   - e-mailadres contactpersoon: [redacted]

6. Adviestraject (data dd-mm-jjjj):
   - ontvangen door DEC: *02-05-2017*
   - aanvraag compleet: *02-05-2017*
   - in vergadering besproken: *09-05-2017*
   - anderszins behandeld: *n.v.t*
   - termijnonderbreking(en) van / tot: *10-05-2017 tot 16-05-2017 en 23-05-2017 tot 31-05-2017*
   - besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen: *n.v.t.*
   - aanpassing aanvraag: *31-05-2017*
   - advies aan CCD: *06-06-2017*

7. Geef aan of de aanvraag is afgestemd met de IvD en deze de instemming heeft van de IvD.
-Datum advies IvD: *02-05-2017*
-Strekking advies IvD: *De IvD geeft aan dat de aanvrager het project met de IvD heeft afgestemd en dat deze de instemming heeft van de IvD.*

8. Eventueel horen van aanvrager: *n.v.t.*

9. Correspondentie met de aanvrager

Vraagronde 1
- Datum: *10-05-2017*
- Strekking gestelde vragen*: 1. Bij de NTS ziet de DEC graag dat er meer uitleg komt over de handelingen met ongerief (3.4) en bij vermindering (4.2). 2. Bijlage 1 onderdeel k, alleen fokdieren waarbij ongerief kan optreden moeten worden vermeld. Bij doden, follow-up metingen en de*

gedragstest licht ongerief vermelden 3. Er moet meer uitleg komen bij de 60% succesrate, hoe ontstaat deze uitval van dieren?

- Datum antwoord: *16-05-2017*
- Strekking antwoorden*: 1. Aangepast, de handelingen zijn afhankelijk van de groep waarin de experimentele dieren zich bevinden. Zo zijn er handelingen waar licht ongerief bij optreedt en handelingen waar matig ongerief bij optreedt. Vermindering: ''We hebben voorafgaand aan dit onderzoek een grote investering gedaan in het opzetten van een non-invasieve screening faciliteit voor kleine proefdieren. Dit betekent dat we de dieren kunnen volgen over de tijd en dat we ze niet steeds per tijd/meetpunt hoeven op te offeren. Met deze technieken kunnen we de functionaliteit van het oog meten en de morfologie van het oog bekijken over de tijd. Zo kunnen we een ontwikkeling goed volgen binnen hetzelfde dier. Dit helpt om het aantal benodigde dieren sterk te verminderen'' 2. Gedaan. 3. De uitval ontstaat* [redacted]
- De antwoorden hebben wel/niet geleid tot aanpassing van de aanvraag: *Ja, de antwoorden hebben geleid tot aanpassing van de aanvraag.*

<u>*Vraagronde 2*</u>

- Datum: *23-05-2017*
- Strekking gestelde vragen*: 1. Graag ziet de DEC nog dat men aangeeft dat door de moeilijkheid van* [redacted] *de kans bestaat dat de procedure niet werkt, noem de verschillende redenen. 2. De aantallen in de NTS en appendix 1 en 3 komen niet overeen, graag aanpassen.*
- Datum antwoord: *31-05-2017*
- Strekking antwoorden*: 1. Aangepast ''If animals cannot be used for follow-up measurements (40% drop-out), the animals will be euthanized. This will be, for example,* [redacted] *'' 2. Dit is aangepast.*
- De antwoorden hebben wel/niet geleid tot aanpassing van de aanvraag: *Ja, de antwoorden hebben geleid tot aanpassing van de aanvraag.*

10. Eventuele adviezen door experts (niet lid van de DEC): *n.v.t.*

B. Beoordeling (adviesvraag en behandeling)

1. *Het project is vergunning plichtig. Het omvat dierproeven in de zin der wet.*
2. *De aanvraag betreft een nieuwe aanvraag.*
3. *De DEC is competent om over deze projectvergunningaanvraag te adviseren. De benodigde expertise op dit wetenschappelijk terrein is aanwezig binnen de DEC.*
4. Geef aan of DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, zijn uitgesloten van de behandeling van de aanvraag en het opstellen van het advies. Indien van toepassing, licht toe waarom: *n.v.t., geen van de leden is betrokken bij dit project.*

C. Beoordeling (inhoud)

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft *(Zie handreiking 'Invulling definitie project'; zie bijlage I voor toelichting en voorbeeld).*

De DEC is van mening dat deze aanvraag een concrete doelstelling heeft en dat het een samenhangend geheel is, het kan getypeerd worden als een project. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. De aanvrager heeft duidelijk de go/no go momenten beschreven. De DEC is er daardoor van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. Gezien het bovenstaande is de DEC van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.

2. Signaleer of er mogelijk tegenstrijdige wetgeving is die het uitvoeren van de proef in de weg zou kunnen staan. Het gaat hier om wetgeving die gericht is op de gezondheid en welzijn van het dier of het voortbestaan van de soort (bijvoorbeeld Wet dieren en Wet Natuurbescherming).: *n.v.t.*

3. *De in de aanvraag aangekruiste doelcategorieën fundamenteel en translationeel onderzoek zijn in overeenstemming met de hoofddoelstelling. De doelstelling is helder omschreven.*

Belangen en waarden

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een directe en reële relatie is tussen beide doelstellingen. Beoordeel of het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld.

Het netvlies (ofwel de retina) is een zenuwlaagje dat de binnenkant van het oog bekleed. Het bestaat uit miljoenen zintuigcellen die het licht opvangen dat het oog binnenkomt. Bij retinale degeneratieve ziektes, zoals Retinitis Pigmentosa (RP) of leeftijd gerelateerde macula degeneratie (AMD), ontstaan zwakke plekken in de retina waardoor het zicht sterk vermindert. Op dit moment bestaan er geen effectieve behandelingen voor AMD en RP, en wanneer het zicht verloren is zijn er geen behandelingsmogelijkheden meer. Daarom is er een sterke behoefte aan nieuwe therapeutische mogelijkheden.

Het directe doel van deze studie is onderzoek doen naar [redacted] *om te kijken of men hiermee de functie van de retina kan herstellen. Het uiteindelijke doel is het ontwikkelen van een nieuwe therapie om verschillende retinale degeneratieve ziektes te kunnen behandelen. Er is een reële relatie tussen deze beide doelstellingen. Het directe doel is nodig om het uiteindelijke doel te bereiken.*

5. Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden welke morele waarden in het geding zijn of bevorderd worden *(Zie Praktische handreiking ETK: Stap 2.B en tabel 1; zie bijlage I voor voorbeeld)*

De belangrijkste belanghebbenden in dit project zijn: de proefdieren, de onderzoekers en de patiënten leidend aan retinale degeneratieve ziektes.

De waarden die voor proefdieren in het geding zijn: De integriteit van de dieren zal worden aangetast omdat de dieren handelingen ondergaan en worden gedood. De waarde van deze proef voor onderzoekers is: Het vergroten van de wetenschappelijke kennis. Waarden die voor patiënten bevorderd worden: De studie draagt naar verwachting bij aan het ontwikkelen van een betere behandeling van patiënten met retinale degeneratie.

6. Is er aanleiding voor de DEC om de in de aanvraag beschreven effecten op het milieu in twijfel te trekken?: *n.v.t*

Proefopzet en haalbaarheid

7. Beoordeel of de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven voldoende gewaarborgd zijn. Licht uw beoordeling toe.

Naar de overtuiging van de DEC beschikt de aanvrager over voldoende expertise en voorzieningen om de projectdoelstelling met de gekozen strategie/aanpak binnen de gevraagde termijn te realiseren. Alle technische voorzieningen die benodigd zijn voor uitvoering van het project zijn voorhanden, evenals voldoende deskundigheid en financiering om het project succesvol uit te voeren. Ervaring binnen het onderzoeksinstituut met vergelijkbaar onderzoek waarborgt het technisch succesvol uitvoeren van de dierexperimenten. Bovendien wordt er nauw samengewerkt met de academische wereld en andere instituten actief binnen dit onderzoeksveld.

8. Beoordeel of het project goed is opgezet, de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstellingen en of de gekozen strategie en experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project. Licht uw beoordeling toe.

De aanvraag heeft een navolgbare opbouw en is naar de mening van de DEC goed opgezet. De voorgestelde experimentele opzet en uitkomstparameters zijn logisch en helder en sluiten aan bij de aangegeven doelstellingen. De DEC acht het reëel om te veronderstellen dat op basis van de resultaten van de voorgenomen reeks experimenten beschreven in het project, nieuwe en/of aanvullende kennis over retinale degeneratieve ziektes zal worden verkregen. De gevraagde looptijd van 5 jaar acht de DEC reëel gezien de opbouw en de financiële ondersteuning.

Welzijn dieren

9. Geef aan of er sprake is van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren: *n.v.t.*

Alle dieren worden gefokt voor het gebruik in dierproeven, er is geen sprake van hergebruik. Er is geen sprake van bedreigde diersoorten, niet-menselijke primaten, zwerfdieren en/of dieren in/uit het wild. De locatie is binnen de instelling van de vergunninghouder. De dieren krijgen adequate verdoving en pijnbestrijding.

10. Geef aan of de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU.

De dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de richtlijn.

11. Beoordeel of het cumulatieve ongerief als gevolg van de dierproeven voor elk dier realistisch is ingeschat en geclassificeerd. Licht uw beoordeling toe.

Het ongerief als gevolg van de dierproeven is naar de mening van de DEC door de aanvragers realistisch ingeschat en geclassificeerd.

Het ongerief is matig bij 100% van de dieren. De dieren ondervinden licht ongerief als gevolg van de follow-up metingen, de gedragstest en het doden. De dieren zullen matig ongerief ondervinden als gevolg van de non-invasieve screening onder anesthesie, [redacted]

[redacted] *en herstel en door het ontwaken uit anesthesie. Bij de* [redacted] *dit is een integraal onderdeel van het retinale degeneratieve ziektemodel en kan helaas niet worden voorkomen. Voordat ernstig ongerief optreedt zullen de humane eindpunten worden toegepast.*

12. Het uitvoeren van dierproeven zal naast het ongerief vaak gepaard gaan met aantasting van de integriteit van het dier. Beschrijf op welke wijze er sprake is van aantasting van integriteit.

De integriteit van de dieren zal worden aangetast door verschillende handelingen; een gedragstest, screening, [redacted] *en omdat de dieren worden gedood. Bij de* [redacted]

13. Beoordeel of de criteria voor humane eindpunten goed zijn gedefinieerd en of goed is ingeschat welk percentage dieren naar verwachting een humaan eindpunt zal bereiken. Licht uw beoordeling toe.

De criteria voor humane eindpunten zijn goed gedefinieerd. De humane eindpunten zullen worden toegepast, wanneer er duidelijke veranderingen zijn in het gewicht en het gedrag van de dieren of wanneer de dieren niet herstellen [redacted] *(kans is <1%).*

Wanneer de dieren niet gebruikt kunnen worden voor de follow-up metingen zullen ze worden gedood. Dit zal zijn wanneer bijvoorbeeld [redacted] *De onderzoekers zijn bezig met het technisch verfijnen* [redacted] *om het percentage van 40% uitval in de toekomst te verlagen. Dit gebeurd in overleg met de IvD.*

3V's

14. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte vervangingsalternatieven zijn. Licht uw beoordeling toe

Het project is in overeenstemming met de vereisten ten aanzien van de <u>vervanging</u> van dierproeven. Het gebruik van proefdiervrije methoden of minder complexe diersoorten is volgens de DEC niet mogelijk.

Voorafgaand aan de dierexperimenten heeft men onderzoek gedaan in celkweek en aan menselijke donorogen. Het betrof hier het karakteriseren, kweken en differentiëren van cellen en weefsels onder diverse omstandigheden. De stap naar experimenten in dieren is echter essentieel voor de ontwikkeling van een nieuwe therapie in mensen. [redacted] *en het daadwerkelijk (verbeteren van) de lokale structuur van het netvlies, kan in celkweek niet worden nagebootst of gemeten, hiervoor is het gebruik van proefdieren noodzakelijk.*

De keuze voor het gebruik van muizen en ratten is naar het oordeel van de DEC gerechtvaardigd. Voor dit onderzoek zijn geaccepteerde en gevalideerde muis- en rat modellen beschikbaar. Muizen en ratten zijn goed vergelijkbaar met de mens, de morfologie en functionaliteit van hun ogen komt overeen met die van mensen. De rat heeft een relatief groot oog wat beter is bij de toepassing [redacted] *. Echter zijn er maar weinig (bestaande genetische) ratmodellen voor oogheelkundig onderzoek. Tegenovergesteld, bij muizen is genetische manipulatie op grote schaal mogelijk waardoor er veel mutante muismodellen bestaan met specifieke genetische afwijkingen, welke specifieke ziekten in het oog nabootsen. Maar de muis heeft daarentegen een relatief klein oog, daarom zal hierbij eerst een pilot worden uitgevoerd om de complexe technieken uit te testen.*

15. Beoordeel of het aantal te gebruiken dieren realistisch is ingeschat en of er een heldere strategie is om ervoor te zorgen dat tijdens het project met zo min mogelijk dieren wordt gewerkt waarmee een betrouwbaar resultaat kan worden verkregen. Licht uw beoordeling toe.

In het project wordt optimaal tegemoetgekomen aan de vereiste van de vermindering van dierproeven.

Door gebruik te maken van het gefaseerd uitvoeren van de experimenten en een poweranalyse wordt voorkomen dat er teveel (of te weinig) dieren worden gebruikt. Dankzij het opzetten van een non-invasieve screening faciliteit voor kleine proefdieren kunnen de onderzoekers de experimentele dieren volgen over de tijd en hoeft men niet per tijd/meetpunt dieren te offeren. Dit helpt om het aantal benodigde dieren sterk te verminderen.

Het maximale aantal proefdieren is proportioneel ten opzichte van de gekozen strategie en de looptijd. De DEC onderschrijft dat het project kan worden uitgevoerd met maximaal 1156 ratten en 480 muizen en acht dit aantal realistisch onderbouwd. Onnodige duplicatie van experimenten wordt voorkomen doordat de onderzoekers goed bekend zijn met het onderzoeksveld en samenwerken met de andere onderzoeksgroepen die vergelijkbaar onderzoek verrichten.

16. Beoordeel of het project in overeenstemming is met de vereiste van verfijning van dierproeven en het project zodanig is opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd.

Het project is in overeenstemming met de vereiste van de verfijning van dierproeven en het project is zo opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd.

Om stress en ongerief te reduceren worden de dieren (zoveel mogelijk) sociaal gehuisvest, en worden de dieren verzorgd en gecontroleerd door bevoegd en bekwaam personeel. Door het gebruik van non-invasieve screening technieken zijn er relatief weinig handelingen die pijn en ongerief veroorzaken. Daarnaast worden adequate anesthesie en pijnstilling gebruikt om het ongerief van de ingrepen tot een minimum te beperken. Bovendien wordt er preventief antibiotica (oogdruppels) toegediend om ooginfecties na behandeling te voorkomen. Voordat ernstig ongerief optreedt, worden de humane eindpunten toegepast.

17. Beoordeel, indien het wettelijk vereist onderzoek betreft, of voldoende aannemelijk is gemaakt dat er geen duplicatie plaats zal vinden en of de aanvrager beschikt over voldoende expertise en informatie om tijdens de uitvoering van het project te voorkomen dat onnodige duplicatie plaatsvindt. Licht uw beoordeling toe: *n.v.t.*

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Geef aan of dieren van beide geslachten in gelijke mate ingezet zullen worden. Indien alleen dieren van één geslacht gebruikt worden, beoordeel of de aanvrager dat in voldoende mate wetenschappelijk heeft onderbouwd.

Dieren van beide geslachten zullen in gelijke mate worden gebruikt.

19. Geef aan of dieren gedood worden in kader van het project (tijdens of na afloop van de dierproef). Indien dieren gedood worden, geef aan of en waarom dit noodzakelijk is voor het behalen van de doelstellingen van het project. Indien dieren gedood worden, geef aan of er een voor de diersoort passende dodingsmethode gebruikt wordt die vermeld staat in bijlage IV van richtlijn 2010/63/EU. Zo niet, beoordeel of

dit in voldoende mate is onderbouwd. Licht uw beoordeling toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd.

Na het doden van de dieren zal men de ogen, het bloed en weefsel verzamelen voor verdere analyse. Er wordt een dodingsmethode uit bijlage IV van richtlijn 2010/63/EU gebruikt.

20. Indien niet-humane primaten, honden, katten of landbouwhuisdieren worden gedood om niet-wetenschappelijke redenen, is herplaatsing of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is: *n.v.t.*

NTS

21. Is de niet-technische samenvatting een evenwichtige weergave van het project en begrijpelijk geformuleerd?

De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd. De NTS voldoet daarmee aan de eisen zoals gesteld in artikel 10.a.1.7 van de Wod.

D. Ethische afweging

1. Benoem de centrale morele vraag

Rechtvaardigen de doeleinden van dit project het voorgestelde gebruik van de dieren?

Bij deze dierproef is de centrale morele vraag: Rechtvaardigt het ontwikkelen van een nieuwe therapie voor de behandeling van retinale degeneratieve ziektes het gebruik van maximaal 1156 ratten en 480 muizen in de dierproef, die daarvan maximaal matig ongerief ondervinden?

2. Weeg voor de verschillende belanghebbenden, zoals beschreven onder C5, de sociale en morele waarden waaraan tegemoet gekomen wordt of die juist in het geding zijn, ten opzichte van elkaar af.

De waarden die voor de proefdieren in het geding zijn: De integriteit van de dieren zal worden aangetast omdat de dieren [redacted] *en handelingen ondergaan en worden gedood. Tijdens de experimenten ondervinden alle dieren matig ongerief. Dit leidt tot veel nadeel voor deze proefdieren. De waarden voor de onderzoekers: voordeel vanwege de kennisontwikkeling. Waarden die voor patiënten bevorderd worden: veel voordeel wanneer het onderzoek zorgt voor de ontwikkeling van een nieuwe therapie voor patiënten met een retinale degeneratieve ziekte.*

De DEC is van mening dat de kennisontwikkeling en lange termijn belangen van de patiënten in dit project zwaarder wegen dan de belangen van de 1156 ratten en 480 muizen die hiervoor als proefdieren gebruikt worden. Er zijn op dit moment geen alternatieven voor deze dierproeven beschikbaar waarmee men de doelstellingen kan bereiken.

3. Beantwoord de centrale morele vraag. Maak voor het beantwoorden van deze vraag gebruik van bovenstaande afweging van morele waarden.

Volgens de DEC rechtvaardigen de doeleinden van dit project het voorgestelde gebruik van dieren. Het directe doel van deze studie is onderzoek doen naar het gebruik [redacted] *voor de behandeling van retinale degeneratie. Het verwachte resultaat, in het kader van het beschikbaar komen van een nieuwe therapie voor patiënten met een retinale degeneratieve ziekte is afgewogen tegen het, als matig geschatte ongerief en het doden van de dieren in de proef.*

De DEC onderschrijft dat de doelstellingen niet zonder het gebruik van proefdieren kunnen worden behaald en acht het gebruik van maximaal maximaal 1156 ratten en 480 muizen en de daarmee samenhangende schade aan deze dieren gerechtvaardigd. Bij het uitvoeren van de dierproeven wordt een adequate invulling gegeven aan de vereisten op het gebied van de vervanging, vermindering en verfijning van de dierproeven. Het project is (1) van substantieel belang en (2) van goede kwaliteit.

(1) Er is sprake van een substantieel maatschappelijk en wetenschappelijk belang. De resultaten van dit onderzoek zullen bijdragen aan de kennis over en de ontwikkeling van een behandeltherapie voor retinale degeneratieve ziektes.

(2) De DEC is van mening dat dit project verantwoord is vanuit wetenschappelijk oogpunt en acht het waarschijnlijk dat op basis van de resultaten van de voorgenomen reeks experimenten beschreven in het project, nieuwe en/of aanvullende kennis zal worden verkregen. De onderzoekers beschikken over ruime ervaring en kennis op het gebied van de te gebruiken methoden en werken nauw samen met andere onderzoeksgroepen. Dit in combinatie met de beschikbare faciliteiten en infrastructuur betekent dat de onderzoekers goed gekwalificeerd en geoutilleerd zijn voor het uitvoeren van het in dit project beschreven onderzoek.

Samenvattend kan worden gesteld dat het als substantieel te kwalificeren maatschappelijk belang en wetenschappelijk belang van het onderzoek naar het oordeel van de DEC opweegt tegen het gebruik van maximaal 1156 ratten en 480 muizen en het daarbij verwachte matige ongerief.

E. Advies

1. Advies aan de CCD

   De DEC adviseert de vergunning te verlenen.

2. Het uitgebrachte advies kan unaniem tot stand zijn gekomen dan wel gebaseerd zijn op een meerderheidsstandpunt in de DEC.

   Het uitgebrachte advies is gebaseerd op consensus.

3. Omschrijf de knelpunten/dilemma's die naar voren zijn gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies zowel binnen als buiten de context van het project *(Zie Praktische handreiking ETK: Stap 4.B)*.

   Er is geen dilemma geconstateerd.

9.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Vrije Universiteit Medisch Centrum te Amsterdam
[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1140020172044

Bijlagen
2

Datum 8 juni 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 7 juni 2017. Het gaat om uw project "Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD1140020172044. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
8 juni 2017
Aanvraagnummer:
AVD1140020172044

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
8 juni 2017
Aanvraagnummer:
AVD1140020172044

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	11400
Naam instelling of organisatie:	Vrije Universiteit Medisch Centrum te Amsterdam
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	64156338
Straat en huisnummer:	De Boelelaan 1117
Postcode en plaats:	1081 HV AMSTERDAM
IBAN:	NL69RAVO1025169891
Tenaamstelling van het rekeningnummer:	VUMC

Gegevens verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	
Afdeling:	
Telefoonnummer:	
E-mailadres:	

Datum:
8 juni 2017
Aanvraagnummer:
AVD1140020172044

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:
Functie:
Afdeling:
Telefoonnummer:
E-mailadres:

[redacted]

Gegevens gemachtigde

Naam:
Adres:
Postcode en plaats:

[redacted]

Wilt u een nieuwe machtiging afgeven?	Nee
Wat mag de gemachtigde doen?	[x] Een projectvergunning aanvragen [x] Een wijziging op een verleende projectvergunning aanvragen [x] Een melding doorgeven op een verleende projectvergunning [ ] Een bezwaarschrift indienen en daarover communiceren met de Centrale Commissie Dierproeven en alle andere handelingen verrichten die nodig zijn voor een goede afwikkeling van het bezwaarschrift [ ] Alle bovenstaande opties

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Datum:
8 juni 2017
Aanvraagnummer:
AVD1140020172044

Over uw project

Geplande startdatum:	1 oktober 2017
Geplande einddatum:	30 september 2022
Titel project:	Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration
Titel niet-technische samenvatting:	De experimentele therapeutische transplantatie van stamcel-retinaal pigment epitheel in diermodellen voor retinale degeneratie
Naam DEC:	DEC Vrije Universiteit / VU Medisch Centrum
Postadres DEC:	[redacted] Amsterdam
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.684,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] Melding Machtiging [x] DEC-advies

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Amsterdam
Datum:	7 juni 2017

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1140020172044

Bijlagen
2

Datum 8 juni 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 8 juni 2017
Vervaldatum: 8 juli 2017
Factuurnummer: 172044

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD1140020172044	€ 1.684,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

11.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Vrije Universiteit Medisch Centrum te Amsterdam
[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1140020172044
Bijlagen
1

Datum 17 juli 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 7 juni 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration" met aanvraagnummer AVD1140020172044. Wij hebben uw aanvraag beoordeeld.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
Met het oog op artikel 10a, lid 1, zijn er algemene voorwaarden gesteld.

U kunt met uw project "Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration" starten. De vergunning wordt afgegeven van 1 oktober 2017 tot en met 30 september 2022.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC Vrije Universiteit / VU Medisch Centrum gevoegd. Dit advies is opgesteld op 6 juni 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden

aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Datum:
17 juli 2017
Aanvraagnummer:
AVD1140020172044

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven

[redacted]

Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Vrije Universiteit Medisch Centrum te Amsterdam
Adres:	De Boelelaan 1117
Postcode en plaats:	1081 HV AMSTERDAM
Deelnemersnummer:	11400

deze projectvergunning voor het tijdvak 1 oktober 2017 tot en met 30 september 2022, voor het project "Experimental therapeutic transplantation of stem-cell derived retinal pigment epithelial cells in animal models for retinal degeneration" met aanvraagnummer AVD1140020172044, volgens advies van Dierexperimentencommissie DEC Vrije Universiteit / VU Medisch Centrum. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is [redacted]
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 7 juni 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 7 juni 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 7 juni 2017;
c Advies van dierexperimentencommissie d.d. 6 juni 2017, ontvangen op 7 juni 2017.

Aanvraagnummer:
AVD1140020172044

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1. Breeding with discomfort and phenotyping				
	Ratten [redacted] /	64	100% Matig	
3.4.4.2 [redacted] n rat (setup of the method)				
	Ratten [redacted] /	140	100% Matig	
3.4.4.3 Experimental transplantation (suspension and scaffold) of stem cell-RPE cells in animal models for retinal degenerative diseases and its safety.				
	Ratten [redacted] /	952	100% Matig	
	Muizen (Mus musculus) /	280	100% Matig	
3.4.4.4 PILOT: Transplantation [redacted] in mouse (setup the method)				
	Muizen (Mus musculus) /	200	100% Matig	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

Aanvraagnummer:
AVD1140020172044

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Centrale Commissie Dierproeven

Aanvraagnummer:
AVD1140020172044

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.