	Inventaris Wob-verzoek W15-11								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS 2015104								
1	Aanvraagformulier				x		x	x	
2	Niet-technische samenvatting	x							
3	Projectvoorstel			x					
4	Bijlage beschrijving dierproeven 1			x					
5	Bijlage beschrijving dierproeven 2			x					
6	Bijlage beschrijving dierproeven 3 oud			x					
7	Bijlage beschrijving dierproeven 4 oud			x					
8	Bijlage beschrijving dierproeven 5 oud			x					
9	Ontvangstbevestiging				x		x	x	
10	Vervolgbrief aanvraag				x		x	x	
11	Verzoek DEC-advies				x		x	x	
12	DEC-advies				x		x	x	
13	Brief antwoorden				x		x	x	
14	Bijlage beschrijving dierproeven 3 nieuw			x					
15	Bijlage beschrijving dierproeven 4 nieuw			x					
16	Bijlage beschrijving dierproeven 5 nieuw			x					
17	Table of groups			x					
18	Advies CCD		x						x
19	Beschikking en vergunning				x		x	x	
20	Mail ontvangstbevestiging 22-5-2015				x		x	x	
21	Mail DEC aanpassing 5-6-2015				x		x	x	
22	Mail aanpassing tekst 11-6-2015				x		x	x	
23	Mail DEC aanpassing 12-6-2015				x		x	x	
24	Mail dubbele betaling 18-6-2015				x		x	x	

26 MEI 2015

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.zbo-ccd.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* 80101 [redacted] KNAW
☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	KNAW
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	5 4 6 6 7 0 8 9

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.

Straat en huisnummer	
Postbus	Postbus 19121
Postcode en plaats	1000GC Amsterdam
IBAN	NL33DEUT0546900054
Tenaamstelling van het rekeningnummer	[redacted]

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted] ☑ Dhr. ☐ Mw.
Functie	Group Leader
Afdeling	Group Regeneration of Sensory-Motor Systems
Telefoonnummer	[redacted]
E-mailadres	[redacted]

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

(Titel) Naam en voorletters	[redacted] ☑ Dhr. ☐ Mw.
Functie	Biotechnicus
Afdeling	Group Regeneration of Sensory-Motor Systems
Telefoonnummer	[redacted]
E-mailadres	[redacted]

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	[redacted]	

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	Startdatum	01-07-2015
	Einddatum	01-07-2020
3.2	Wat is de titel van het project?	Developing strategies to promote repair or plasticity of the central and peripheral
3.3	Wat is de titel van de niet-technische samenvatting?	Ontwikkeling van strategieën om de regeneratie van zenuwweefsel te bevorderen
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC: DEC-KNAW
	Postadres	[redacted] Amsterdam
	E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

- ☑ Nieuwe aanvraag Projectvergunning € 741,00 Lege
- ☐ Wijziging € Lege

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.

- ☑ Via een eenmalige incasso
- ☐ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

- ☒ Projectvoorstel
- ☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

- ☐ Melding Machtiging
- ☒ Appendixen 5 maal

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 1.6). 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	Directeur Instituten KNAW
Plaats	Amsterdam
Datum	18-05-2015
Handtekening	[redacted]

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.zbo-ccd.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'.	NVWA80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
1.3	Provide the title of the project.	Developing strategies to promote repair or plasticity of the central and peripheral nervous system

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

X Basic research

☐ Translational or applied research

☐ Research into environmental protection in the interest of human or animal health or welfare

☐ 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.

Dysfunction of the brain or spinal cord (collectively referred to as the central nervous system [CNS]) or of the peripheral nervous system (PNS) due to neural degeneration or traumatic injury affects millions of people worldwide and has a significant physical, emotional, and socio-economical impact. There are many different causes for neural degeneration or injury ranging from genetic causes, toxic insults, traffic and sports-related incidents, community violence and work-related incidents. Nervous system degeneration primarily entails the death of specific populations of neurons themselves (e.g. the loss of dopaminergic neurons in patients with Parkinson's disease) and/or the interruption of nerve projections and the associated loss of synaptic contacts (e.g. in patients with brain, spinal cord or peripheral nerve injury). When nerve cells are lost, these cells are not replaced by new cells (as opposed to cells in many other organs). In the CNS damaged, interrupted nerve tracts normally do not regrow, while in the PNS injured nerve tracts do regrow but only to some extend and after severe lesions most nerve fibers do not re-innervate the target. The failure to regenerate is due to the induction of the expression of growth inhibitory factors, for instance at the site of the injury (in the neural scar formed after a CNS lesion), and the lack of the production of pro-regenerative molecules (e.g. neurotrophic factors or transcription factors). In the injured CNS the balance shifts to growth inhibition and, therefore, regeneration is very poor in the CNS. However, some recovery of function may occur due to the induction of growth of intact neighboring axon pathways. This process is usually referred to as plasticity and is also an important mechanism for partial recovery of function and repair. In the PNS the situation is slightly better than in the CNS, e.g. Schwann cells in an injured peripheral nerve do support nerve fiber growth, but after longer times of denervation these cells deteriorate and do not support growth anymore.

Currently there are no effective treatments for patients that have sustained injury to the CNS. For instance, Parkinson patients are treated pharmacologically with L-DOPA to supplement the loss of dopamine. This treatment temporally alleviates some of the symptoms of the disease but it is not a regenerative treatment. Patients with a lesion of the PNS can be treated by a neurosurgeon, however, regeneration and functional recovery of a nerve following surgical intervention is almost never complete. Therefore, what is urgently needed is more fundamental knowledge on (i) which molecules and genes determine the loss of neurons, (ii) which factors control in the outgrowth of neuronal projections, and (iii) which factors limit the regeneration of injured neurons. This knowledge will be tested for their potential to be used as genuine regenerative treatments that (1) promote neuronal survival in order to prevent neurons from degeneration, (2) promote nerve fiber outgrowth and the formation of new synaptic connections of lesioned or spared nerve tracts by adding growth promoting factors or the removal of inhibitory factors. We focus our research both on genuine regeneration of injured nerve tract and on structural remodeling of spared nerve tracts (also referred to in the field as structural plasticity) because structural remodeling of the intact fibers may also have a significant positive impact on functional recovery. For many of our project we have used human nervous tissue as the starting material of our screens and as tissue to start to refine our gene transfer methods.

There is general agreement in the research field of neurodegeneration and regeneration that the current neurosurgical intervention strategies have reached optimal refinement. Therefore these interventions will not lead to the level of repair that is required for patients with nervous system injury to allow a return to a normal life. Hence the long-term aim of the research in this field should be on developing effective regenerative treatments. For this it is essential to identify factors (genes, molecules, cell types) that are pivotal in the survival of neurons, the regeneration of injured nerve projections and the formation of new synapses. We and others have already identified two cell types *[Schwann cells (SCs) and Olfactory ensheathing glia cells (OEGs); reviewed in Roet and Verhaagen, Experimental Neurology 2014]* and several molecules that either promote neuronal survival, axon regeneration or plasticity *[growth factors, wnts, transcription factors; reviewed in Fagoe et. al. 2014]* or hamper axon regeneration or plasticity *[extracellular matrix molecules, e.g. semaphorins; reviewed in Mecollari et. al. Frontier in Neuroscience 2014].* Some of these cell types and factors have subsequentially been tested for their neuroprotective or growth-promoting effects in lesion models in experimental animals. Specific examples of work from our own laboratory include the profound pro-regenerative effect of transplanted, genetically modified, OEGs in a spinal cord lesion *(Ruitenberg et.al. Journal of Neuroscience 2003)*, of the growth factor BDNF on the survival of rubrospinal neurons *(Ruitenberg et al Neurobiology of Disease 2004)* and of the growth factor GDNF on motor axon outgrowth *(Eggers et. al. Molecular Cellular Neuroscience 2008, Hoyng et. al. Experimental Neurology 2014a)*. Recent, as yet unpublished, discoveries from our group include the observation that the signaling molecule Wnt5a promotes nerve fiber outgrowth *(van Vliet, unpublished)* and that functional neutralisation of Semaphorin3A (a molecule obstructing regeneration) promotes repair of the injured spinal cord *(Mecollari, unpublished)* and enhances plasticity in the brain *(Vo et. al. Molecular and Cellular Neuroscience 2013, and unpublished observations in collaboration with Daniela Carulli and Tommasso Pizzurosso, Italy)*. Moreover we have identified molecules that induce degeneration of neurons, specifically the dopaminergic neurons that die in Parkinson's disease (*Bossers et al Brain Pathology 2009; Korecka and Moloney et al unpublished*).
As a means to express pro-regenerative molecules in damaged neural tissue in order to study their functional involvement, we have developed advanced and innovative gene transfer strategies (based on adeno-associated and lentiviral vectors, some of which have clinical potential; *reviewed in Mason et.al. Current Gene Therapy 2011)*. Gene transfer with viral vectors is chosen as a primary approach because it is a very powerful method to locally express a gene and study its function in the injured nervous system. Moreover eventually this strategy may be applicatble clinically since in the last 10 years an the adeno-associated viral (AAV) vector has gained increasing acceptance as a clinical gene therapy platform. Viral vector-mediated gene transfer was optimized for gene transfer in injured neurons by testing which serotype *(Mason et.al. Molecular Therapy 2010, Blits et.al. J. Neuroscience Methods 2011; Korecka et. al. Viral vectors 2011)* and route of delivery *(Fagoe et.al. Neuromethods 2015)* was the best. In addition, we also combined tissue transplantation with gene transfer e.g. in a study where we used genetically modified nerve autografts to repair injured peripheral nerves *(Hoyng et.al. Experimental Neurology 2014)*. Although gene therapy is in our view a very powerful way to study gene function, it may also be a clinically applicable strategy to promote repair the use of small molecules (inhibitors, agonist; that is pharmacological intervention). This is certainly an option that we want to pursue if this would be more rational.

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 main aim of this project is to unravel the fundamental cell and molecular biological mechanisms that underlie the failure of the nervous system to repair itself. Specifically the project aims to identify molecules that promote or inhibit the neuroregeneration process, to overexpress or neutralize these molecules and to study the effects of these interventions on the recovery process.
The laboratory and scientific infrastructure needed is available at the Netherlands Institute for Neuroscience which makes this research highly feasible. In addition to the scientific achievements of the group summarized above, the group has a long-term internationally recognized track record in

neuroregeneration research. This is illustrated by the fact that the group published over 190 papers on this topic, received national and international funding and was positively evaluated by the KNAW-audit committee in 2012. The group has been part of several European consortia and has international collaborations with groups in e.g. Cambridge, Turin, and Perth.
Taken together, and based on the available data, know-how, and infrastructure as summarized above, we expect that in the next five years it will be realistic to firmly establish the role of at least three and perhaps six new molecular targets in the neural repair process. We also collabrate with clinicians, e.g. at the department of neurosurgery at the LUMC, to allow translation of our research to the clinic.

3.3 Relevance

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

This project is important for two reasons: 1. It results in fundamental insights into the mechanisms that inhibit or promote regeneration, and 2. These results form the basis for repair strategies that will eventually be applied to promote repair in patients.

Apart from pharmacological treatment (e.g. with L-DOPA in Parkinson patients which results in temporary relief of symptoms) or neurosurgical repair of an injured peripheral nerve (which results mostly in only partial recovery of function) there are no effective treatments available for patients with nervous system degeneration/injury. Individuals with neural injury therefore suffer from a life-long disability and many are usually dependent on outside care and/or are bound to a wheelchair. There is an urgent need for treatments that promote nervous system repair and full functional recovery.

In the current project we develop strategies that have the aim to promote neuroprotection and/or neuroregeneration with the long-term goal to lead to medically applicable treatment options for patients with brain, spinal cord or peripheral nerve injuries. These regenerative treatments will be beneficial for individual patients and for society as a whole because effective treatments may allow patients to rehabilitate and improve their quality of life.

3.4 Research strategy

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

To achieve the aims described above we make use of a four step strategy (our "target discovery pipeline") developed by us that includes (1) large gene expression and proteomics screens on control (non-damaged) or damaged human neural tissue or on control and damaged tissue of experimental animals to identify molecules involved in the neural repair process, (2) bioinformatical analysis of the resulting gene and protein data sets in combination with published datasets to identify the most promising targets, (3) to perform bioassays for neuronal survival, axon outgrowth and synapse formation to confirm a functional role of the identified candidate targets using primary neural cells, and finally (4) gene and cell therapy studies in (transgenic) animal models of neurodegeneration and injury to test the mechanism of action and/or effectiveness of a final set of most promising targets. For this, we have developed and tested advanced viral vector-mediated gene transfer technology that allows us to express genes in vivo in injured neurons or glia cells. We are also in the process of developing regulatable viral vectors that are based on a novel immune-inert transactivator that allows antibiotic-mediated control over transgene expression in vivo (Hoyng et al Experimental Neurology 2014b). Our approach has shown to result in valuable fundamental knowledge and form the foundation for novel, potential repair strategies in patients.

Thus, the overall strategy of this project consists of a number of distinct steps aiming at target discovery for neural protection and repair using molecular screens and bioinformatics from published data sets and from data obtained from our own models (procedure 3.4.4.1), target validation in vitro by means of

bioassays (procedure 3.4.4.2), and investigation of target efficacy in vivo in (transgenic) animal models (procedures 3.4.4.3; 3.4.4.4; 3.4.4.5) of neural degeneration and regeneration. We refer to this strategy as our "target-discovery strategy". Below we will describe how we execute each of these steps and what is required in terms of types of animal experiments.

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.

Molecular screens. The first step in our target finding approach are molecular screens, using microarray or RNA-sequencing, which are performed on unaffected control neural cells and on injured neural cells. The comparison yields insights into the molecular differences between intact, non-injured neural cells and injured neural cells. This step is performed on several different sources of tissue, including human nervous system tissue obtained from the Netherlands Brain Bank, from the operation theatre (both do not require experimental animals), or from tissue obtained from experimental animals after a lesion of e.g. a spinal cord or a peripheral nerve lesion or from transgenic animals. Animal experiments are needed to obtain tissue from animals with or without a neural lesion (procedure 3.4.4.1; for neural lesions see "injury models" below).
Bioassays. Bioassays are essential for two reasons. First, the molecular screens usually result in many different interesting targets and only the most promising molecules need to be selected for testing in injury models. We have large-scale bioassays to measure the effect of overexpression or knockdown of target on e.g. neuronal survival and axon outgrowth, two processes directly relevant to neuroprotection and repair. Second, viral vectors used for gene transfer have to be tested for efficacy on primary neurons or glia cells before any application in vivo. For both applications we need to culture primary neurons or glia cells derived from rat or mouse embryo's or adult mice or rats (procedure 3.4.4.2).
Injury models. It is important to test our targets under different circumstances because certain parts of the nervous system regenerate to a certain extend (e.g. the peripheral nerve) while other parts (the spinal cord) do hardly regenerate. Therefore we need different types of injury. The efficacy of a selected target is determined either in an animal model for neural injury (in case we expect an effect on the regeneration process) or in a naïve animal (in case we hypothesize that a molecular target is involved in neurodegeneration or synapse loss as is e.g. the case for some chemorepulsive proteins). The lesion is produced e.g. by means of transection of a nerve tract in the spinal cord (e.g. the dorsal column or the corticospinal tract) or transection of a peripheral nerve. Type of animal experiment: lesioning of the brain, spinal cord or peripheral nerve is part of procedures 3.4.4.3, 3.4.4.4, 3.4.4.5.
Viral vector or cell-mediated gene delivery of a target. Targets identified in the molecular screens and functionally validated in bioassays are delivered to the nervous system by means of viral vectors (direct in vivo gene transfer) or by means of cell transplants that have been exposed to a viral vector in vitro ("ex vivo gene transfer"). The animal experiment required here is: Stereotactic injection of a viral vector or of cells in a specific brain nucleus, in the spinal cord, in a spinal ganglion, or in the peripheral nerve or the muscle. The chosen injection site is dependent on the specific target and on the goal of the experiment (procedures 3.4.4.3 - functional and histological analysis). Moreover we need to test the performance of most new produced batches of viral vectors in vivo on a small number of animals (procedure 3.4.4.4).
In the ideal situation, the efficacy of the viral vector or cell-mediated gene delivery of a target will be carried out in one of the injury models to test the effect of a selected target on neuronal degeneration or regeneration. However, for some experiments WT or transgenic mice may be used, for instance when a target is implicated in neuronal degeneration or neuroplasticity. To test the effects of the application of a target two main read-out parameters are used in parallel: functional and morphological tests (part of procedure 3.4.4.4).
Functional analysis. The efficacy of a treatment may evaluated by means of electrophysiological and/or functional approaches. With electrophysiology, the return of compound motor action potentials (CMAPs) and spinal evoked potentials are evaluated in time after the injury. Functional behavioral tests include the narrow beam test, gridwalk test, grip test, open field test, rope test, cylinder test, pole jump test, kinematic gait analysis test, catwalk gait analysis and foot flick test (part of procedure 3.4.4.3.).
Morphological analysis. For this, tissue for histological analysis is obtained following perfusion fixation of an experimental animal at different times after inflicting the injury. The efficacy of a particular treatment or target is analyzed at the level of cell survival, degree of axon outgrowth, scar formation and synapse formation. Type of animal experiment: Perfusion of rats and mice. (part of procedure 3.4.4.3.).

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.

In this project we identify and characterize target molecules that promote or inhibit repair of the CNS or PNS using bioassays and in vivo models. Some targets have been identified based on previous discoveries by ourselves or of others, e.g. we work on a number of growth factors (NGF, BDNF, GDNF) and repulsive guidance molecules (Semaphorins) in the context of regeneration, structural plasticity and synapse formation. The "target discovery strategy" described above has already served to identify a number of new targets by ourselves. Typically, this pipeline consist of 4 steps that logically follow each other and progresses from "target identification" to "efficacy studies" in an animal model. For each target a go/no go decision is made whether or not the target should be studied at the next step. Primary target identification is based on human tissue obtained from the Netherlands Brain Bank or from the operation theatre obtained in collaboration with neurosurgeons. In some cases we perform genome wide gene expression studies on tissue from (transgenic) mice or rats using our injury models.

The logical structure of these different phases is best illustrated by an example from our recent research: Step 1/2: screening (step 1) and bioinformatics (step 2) - the protein Wnt5a was selected from a small group of highly upregulated molecules in a screen of injured human peripheral nerve tissue that was removed by the neurosurgeon during a nerve repair operation. Step 3: The effect of Wnt5a upregulation was studied in primary neuronal cell cultures and we found that Wnt5a significantly promotes neurite outgrowth. Therefore, Wnt5a was taken to step 4: testing of the effect of Wnt5a overexpression in vivo in a rat peripheral nerve injury model using both functional and histological read-outs. Moreover, in a Wnt5a knock-out mice introduced in the Netherlands Institute for Neuroscience from a group in Japan (Hiroaki Honda, Hiroshima University) we are currently studying in vivo effects of Wnt5a on axon regeneration. Taken together, for each target go/no go decisions are made based on the performance in each specific stage of the project as illustrated above for Wnt5a.

It is important to note, that there is some overlap between the animal studies described in this project and those in earlier DEC-approved protocols. After a license for this project has been obtained, all experiments will formally be executed under this new license.

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	Molecular screens: Sacrifice of mice or rats following a lesion and/or intervention with a viral vector to obtain neural tissue for molecular screens
2	Ex vivo bioassays: Sacrifice of embryos of mice or rats or of adult mice or rats to obtain tissues for cell culture and bioassays
3	Injury models (functional and histological analysis): Injection of cells or viral vectors in lesioned animals
4	Testing of the quality of viral vector batches
5	Monitoring and testing of novel genetically modified mice
6	
7	
8	
9	
10	

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.zbo-ccd.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'.	NVWA 80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
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: Molecular screens: Sacrifice of mice or rats following a lesion and/or intervention with a viral vector to obtain neural tissue for molecular screens

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.

A primary aim of our research is to unravel new mechanisms that govern or hamper neuroregeneration or neuroplasticity. To achieve this we perform, in addition to analysis of patient material, molecular screens on neural tissue after a lesion to identify or mechanistically validate molecules that are potentially involved in the regeneration process or in the processes that hamper regeneration.

The brain, spinal cord or peripheral nerve lesions will be performed by means of well-established neurosurgical procedures. The types of lesion are chosen because they represent clinically relevant injuries. In different parts of the CNS different processes underlie degeneration and regeneration and it is therefore important to study different parts of the CNS (brain, spinal cord, spinal ganglion, peripheral nerve) and innervated target muscles. In some instances we will perform a lesion and inject a viral vector encoding for a gene of interest ("a target") or a molecule of interest (e.g. a transcription factor, Wnt5a or Semaphorin3A). At a particular post-lesion interval animals will be killed and tissue will be dissected out for a molecular screen, e.g. a microarray screen, RNA-sequencing or proteomics analysis. This specific aim is to provide quantitative insight in the changes in mRNA or protein levels that occur in neural tissue after a lesion and/or after applying a specific treatment (e.g. viral vector-mediated gene transfer of a specific target gene).

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

This procedure can consist of the following steps:

1) *Application of a lesion to the brain, spinal cord or peripheral nerve*. Under adequate anaesthesia and perioperative analgesia, the brain, spinal cord, or peripheral nerve is exposed and a unilateral or bilateral lesion is made by means of damaging a nerve tract.
    a) *Brain*: injection of a neurotoxin systemically (I.P.) or intracranially, or by means of inflicting mechanical injury with micro-scissors *(max 1x).*
    b) *Spinal cord*: At cervical or thoracic or lumbar level, access to the spinal cord is gained and a uni- or bilateral lesion of the rubrospinal tract, corticospinal tract, dorsal column or dorsolateral columns is performed by means of microscissors *(max 1x).*
    c) *Peripheral nerve*: After gaining access to the peripheral nerve lesion site, unilateral crush or transection or spinal root avulsion is performed *(max 1x).*
2) *(Optional) Viral vector or cell-mediated gene delivery of a target or injection of the target molecule itself.* Under adequate anaesthesia and postoperative analgesia a (stereotaxic) injection of a viral vector (a control vector encoding GFP or an experimental vector encoding a target gene) or of cells (control cells expressing GFP or cells expressing an experimental target gene), or of tissue grafts (virally transduced or not), in a specific brain area, spinal cord, spinal ganglion, peripheral nerve, or target muscle is performed.
    a) In 80% of the experiments, this procedure is performed simultaneously with the lesioning procedure resulting in only one exposure of the animal to surgery (max 1x).
    b) In 20% of the experiments, the target expression needs to be present prior- or after the lesion has been applied resulting in a separate surgery (max 1x).
3) The animals will be sacrificed at defined time point ranging between 6 hours and 2 months after the lesion by administration of an overdose of barbiturate (I.P) or via CO2/O2 sedation followed by decapitation. Relevant tissues will be dissected out and will be processed for the molecular screen.

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

Parameters can be qualitative or quantitative. In the case of quantitative analysis, prior to performing an experiment we perform a power analysis (e.g. a power analysis). Many years of experience will allow us to do this in an efficient and reliable way.

B. The animals

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

Mice: genetically modified and wild type; The mice are obtained from our own breedings or from a commercial licensed breeder.
Mice (adult): 240.
Rat (adult): 240.
The estimate of the total number of animals is primarily based on our experience over the past 5 years. This number of experiments and therefore the number of animals is dependent upon the number of involved researchers which is strongly dependent on the available funding. However, a general estimate for the total number of rats and mice is as follows. A typical experiment will consist of 4 groups: a control group and 3 groups at 3 post-lesion time points. Each group consists on average of 6 animals. Total number of animals for typical experiment is 24. We expect to do 2 of these experiments per year on mice (48 mice/year) and 2 of these experiments on rats (48 rats/year)which results in a total of approximately 240 mice and 240 rats over a period of 5 years.
Mice will be used when we want to measure gene and/or protein expression differences in a situation where we want to compare genetically modified (e.g. a knock-out for a target) versus wild type mice. Rats will be used for all other experiments since the size of rats allows more precise surgical lesion procedures and viral vector injections.
Importantly, before we start our experiments we will write an application to the IvD. In this application we will exactly describe (among others) which considerations, facts and results have led to the proposition of the experiments, which specific question(s) we are trying to answer with the proposed animal experiments and what the ultimate goal is for the proposed experiments. Moreover we will describe in full detail the experimental design, argue the number of animals in the experiments, describe human endpoints, alternatives, and the nature of discomfort. Experiments will only be started upon IvD approval.

C. Re-use

Will the animals be re-used?

☒ 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'?

☐ 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.

The procedures described in this project are based on a large body of scientific- and experimental experience in both rats and mice. Testing of the intervention-techniques will be performed *in-silico* and *in-vitro* as much as possible prior to performing an animal experiment. However, to fully understand and study the proposed mechanisms/targets in the context of neurodegeneration/degeneration, animal studies are necessary because of the complexity of the processes occurring following a lesion.
These studies are worldwide conducted in both rat and mouse, making translation and extrapolation of data between the different research-groups worldwide feasible.
Experiments will be executed in succession and, if needed, small explorative studies will be performed to provide the necessary insight in variation and expected results. On basis of our previous work and experience and explorative pilot experiments, statistical analysis can be performed to determine the

minimum number of animals needed to obtain scientific valuable data.
In the case when transgenic mice are used; presence of an existing mouse line is checked, and/or an attempt is made to obtain the target transgenic mouse in as little breeding steps as possible, reducing breeding time and animals. In addition, mice with inducible alleles will be used whenever possible, resulting in a normal phenotype until induction with tamoxifen.

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

All surgical procedures resulting in animal suffering or pain will be performed under adequate anaesthesia and analgesia. Close postoperative monitoring will be performed and humane endpoints applied when indicated. All available resources to reduce pain, fear or suffering will be employed.
Procedures will only be performed by competent personnel, as mandatory.
Adverse environmental effects are not present.
All rats and mice will be socially housed with the appropriate environmental enrichment under strict DM2 (if a viral vector is injected) conditions, or at DM1 (if transgenic mice are used).

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.

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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.

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

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

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

analgesia is applied prophylactic and when indicated.

I. Other aspects compromising the welfare of the animals

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

As a result of the applied loss of innervation/ neurodegeneration, a behavioral response consisting of excessive licking and biting at the affected area might occur. In time this can lead to tissue damage (autotomy). In our experience this behavior occurs mainly between 2-8 weeks post lesion and is *not* present in all types of lesions. Spinal cord and dorsal root lesions are the types of lesions where this occurs most frequently.
It is expected that in such a specific experiment 2-5% of all animals will be experiencing these adverse effect at various degrees.
All animals will be frequently monitored for possible side effects.
Animals exhibiting any unexpected phenotype resulting in constitutional discomfort will be killed within a day.

Explain why these effects may emerge.

The precise mechanism behind autotomy is still largely unknown. This behaviour only occurs in an denervated area/limb and is in the majority of cases transient.

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

Animals will be monitored daily and if possible treatment will be initiated (topically or systemic).

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.

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

Weight loss (>20% weight loss compared to pre-operative bodyweight), abnormal/unexpected behaviour and/or posture, general signs of illness and/or discomfort. Autotomy. The animals will never experience more than moderate discomfort.

Indicate the likely incidence.

Expected 2-5 % within time frame of specific experiment in 33% of the experiments.

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').

Moderate in 100%. In most of the cases this discomfort is transient (1-2 days).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

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

The rats or mice will be killed for histological analysis.

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.

☒ 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.zbo-ccd.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'.	NVWA 80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience

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	Type of animal procedure
# 2	Sacrifice of embryos of rats or mice or of adult rats or mice to obtain tissues for cell culture and bioassays.

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.

This project invests in the elucidation of the fundamental cell and molecular biological mechanisms that underlie the failure of the nervous system to repair itself. This fundamental knowledge is essential to identify the key molecules (also referred to as "targets") that regulate crucial aspects (neuronal survival, neurite outgrowth, synapse formation) of degeneration, regeneration, and functional recovery.

In order to identify and screen for targets and perform a pre-screening on the function and effect of these targets prior to *in-vivo* studies, molecular screens and bioassays are performed *in-vitro* using primary cell cultures obtained from rat or mice (embryos and adults) being either TG or WT. Alternatives (i.e. cell lines) are in most instances not compatible with the research question due to altered gene expression patterns resulting in the lack - or presence- of specific receptors (for example cell lines have been proven to be insensitive to semaphorins).
Animals will be killed according to Annex IV of directive 2010/63/EU and tissues will be harvested for further culturing.
Dependent on the research question and assay performed, cells are harvested from rats or mice (WT/ TG) from embryos, pups (P1-P7) or adults. To be able to answer specific research questions, primary cells from transgenic mice that are (conditional) knockout, mutant or transgenic for genes that might be associated with the above mentioned targets are needed.
These cultures are used for:

- Bioassays (outgrowth-, migration-, viability-, repulsion-, collapse- assays)
- Immunocytochemical- or in-situ hybridisation staining
- Biochemical analysis (ELISA, FACS, Microarray, RNA/ DNA/ Protein extraction).
- Manipulation of the cells (via viral- or plasmids driven gene expression, pharmacologically) and subsequent analysis using the above mentioned techniques.

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

This procedure will consist of the following steps:

- The animals will be sacrificed by administration of an overdose of barbiturate (I.P), or via CO2/O2 sedation followed by decapitation.
    - In the case of embryonic tissues: pregnant mothers will be euthanized. Embryos are quickly taken from the uterus and placed on (but not in direct contact with) melting ice water, followed by decapitation. Brains will be isolated and kept cool and tissue will be harvested for culturing.
    - In the case of pups: Pups are placed on (but not in direct contact with) melting ice water, followed by decapitation. Brains will be isolated and kept cool and tissue will be harvested for culturing.
    - In the case of adult tissues: animals will be euthanized and tissue will be harvested for culturing.
- Tissues that are harvested include, but are not limited to: Brain, meninges, peripheral nerve, spinal cord, spinal dorsal root ganglia (DRG, SCG), muscle, skin.

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

Quantitative analysis: prior to performing an experiment we perform statistical analysis (a power analysis) to ensure that we use the minimum number of animals per group that will be statistically sound and biological relevant.
Qualitative analysis: The number of animals is based upon our large experience in the past. This concerns knowledge about the total number of cells that can be obtained from specific tissues per animal and the expansion of these cells during passaging. Experimental design further dictates the number of cells needed. Finally, experiments are performed sequentially resulting in increasing knowledge about the variation in the target and control groups.

B. The animals

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

The estimate of the total number of animals is primarily based on our experience over the past 5 years. This number of experiments and therefore the number of animals is dependent upon the number of involved researchers which is strongly dependent on the available funding. However, in general, an estimate for the total number of rats and mice is as follows:
Rat and mice embryo's: an average of 25 pregnant mice and 25 rat pregnant mothers (litters) is needed per year to perform primary cultures and bioassays

(e.g. dorsal root ganglia) described in this protocol. One litter generally provides enough cells from any of the proposed tissues, to perform an in-vitro study (including experimental groups, positive- negative- and biological controls). For 5 years, 125 rat and 125 mice pregnant mothers, with an average of 6 to 8 pups per mother. *For 5 years that amounts to maximally 1000 rat embryos and 1000 mouse embryos (and the 125 rat and 125 mice pregnant mothers).*

Early post-natal rats and mice (P1 to P7): an average of 75 rat pups and 75 mouse pups is required per year to perform primary cultures and bioassays using post-natal cells (e.g. cerebellar granule cells, cortical neurons). *For 5 years a total of 375 rats and 375 mice pups are required.*

Adult rats and mice: an average of 20 adult rats and 20 adult mice is necessary to set up primary cultures and bioassays using adult neurons (mostly dorsal root ganglion neurons). *For 5 years a total of 100 adult rats and 100 adult mice are required.*

C. Re-use

Will the animals be re-used?

☒ 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'?

☐ 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.

The procedures described in this project are based on a large body of scientific- and experimental experience in both rats and mice. Testing of the intervention-techniques will be performed *in-silico* and *in-vitro* as much as possible prior to performing an animal experiment.
In case an alternative (i.e. a cell line) is available that is applicable and will answer the research question, these are the primary choice.
Optimal use is made from each animal killed by harvesting the maximum amount of tissue from different organs.
These studies are conducted in both rat and mouse worldwide, making translation and extrapolation of data between research-groups more feasible.
Experiments will be executed in succession and, if needed, small explorative studies will be performed to provide the necessary insight in variation and expected results. On basis of this previous work and experience, statistical analysis can be performed to determine the minimal number cells/ culture dishes and thus, the minimal number of animals needed to obtain valuable data.
To reduce inter- and intra-assay variability we will only use well-established reagents and protocols during the in-vitro procedures.

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

All procedures resulting in animal suffering or pain will be performed under adequate anaesthesia. All available resources to reduce pain, fear or suffering will be employed.
Experiments will be done sequentially. Whenever possible small scaled pilot studies will be performed with the minimal number of animals.
Procedures will only be performed by competent personnel, as mandatory.

Adverse environmental effects are not present.
All rats and mice will be housed under strict DM1 and SPF regulations.

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.

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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.

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

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

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

I. Other aspects compromising the welfare of the animals

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

No other adverse effects are expected. Animals will experience normal housing conditions without additional handling untill they are killed.

Explain why these effects may emerge.

Not applicable

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

Not applicable

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.

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

Indicate the likely incidence.

Expected 0% within time frame of the experiment

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').

mild 100%

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

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

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.

☒ 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.zbo-ccd.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'.	NVWA 80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
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: Injury models (functional and histological analysis): Injection of cells or viral vectors in lesioned animals.

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.

Our aim is to determine whether our identified target molecules and intervention methods (i.e. viral vector or cell-mediated gene delivery of a target, by using transgenic mice with overexpression or knock-down of a target) will improve both anatomical and functional recovery following a lesion to the brain, spinal cord, or peripheral nerve. Therefore, following a lesion we will perform several non-invasive function tests and/or an electrophysiological test on each

animal at several time points after induction of the lesion and the therapeutic intervention. This will provide insight in the full spectrum of the induced functional deficit and the degree of subsequent recovery of function per animal in time.
The brain, spinal cord or peripheral nerve lesions will be made by means of well-established surgical procedures. These lesion types are chosen because they represent clinically relevant injuries in which we can test the efficacy of a specific intervention. In different parts of the CNS different processes underlie degeneration and regeneration and it is therefore important to study different parts of the CNS (brain, spinal cord, spinal ganglion, peripheral nerve) and innervated target muscles.
The cells or viral vectors will be injected via small needles in the area of study.
Activation or inhibition of transgenes encoded in the viral vectors or transgene mouse via pharmaceuticals (e.g. doxycycline, tamoxifen) might be necessary to regulate transgene expression in time.
Intravenous application of a substrate (e.g. luciferine) might be necessary to perform an imaging study and visualize viral vector mediated expression of the reporter gene luciferase.
Assessment of recovery of function is obtained by performing multiple function tests at multiple time points.
Animals might be injected under anaesthesia with an antero/retrograde tracer prior to killing the animals to allow histological assessment of regeneration/sprouting process at the intermediate or the final stages of recovery.
At the end of the experiments in all cases the animals will be killed and tissues will be harvested for further analysis, allowing direct correlation between the individual degree of function recovery and histological parameters. These tissues can be subjected to: histological sectioning followed by immunohistochemical- or in-situ hybridisation staining or biochemical analysis (ELISA, FACS, Microarray, RNA/ DNA/ Protein extraction).

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

This procedure will consist of the following steps:

1) *Pre-training and baseline measurement of the animals using behavioural function tests*. Acclimatization of the animals to both the handling and testing-environment will result in reliable baseline data with little variation. This step involves:
    a. Pre-training on our non-invasive function tests performed on freely moving animals to obtain baseline values *(max: 1x exposure/ test/ day, for 2 weeks).* In general, animals performing one or more non-invasive function tests including, for example: 1) cross a beam, rope or narrow corridor, walking from platform to platform without being forced. 2) Grip a horizontal bar and pull after which the maximum applied force is measured. 3) Walk in an open field or cylinder while being observed by the researcher who is scoring naturally behaviour 'events'. Dependent on the aim of the experiment the relevant test(s) and variants thereof will be selected and applied.
2) Baseline measurement of electrophysiological (CMAP) measurement under adequate anaesthesia. *(max: 2x)*
3) *Application of a lesion to the brain, spinal cord or peripheral nerve*. Under adequate anaesthesia and postoperative analgesia, the skull, spinal cord or peripheral nerve is exposed and a unilateral or bilateral lesion is made by means of damaging of a nerve tract.
    a. *Brain*: injection of a toxin systemically (I.P.) or intracranially, or by means of mechanical injury with microscissors *(max 1x).*
    b. *Spinal cord*: At cervical or thoracic or lumbar level, access to the spinal cord is gained and a uni- or bilateral lesion of the rubrospinal tract, corticospinal tract, dorsal column or dorsolateral columns is performed by means of microscissors *(max 1x).*
    c. *Peripheral nerve*: After gaining access to the peripheral nerve lesion site, unilateral crush or transection or spinal root avulsion is performed. *(max 1x).*
4) *Viral vector or cell-mediated gene delivery of a target.* Under adequate anaesthesia and postoperative analgesia a (stereotaxic) injection of a viral vector (an control vector encoding GFP or 'repair experiments' with an experimental vector encoding a target gene) or of cells (control cells expressing GFP or cells expressing an experimental target gene), or of tissue grafts (virally transduced or not), in a specific brain area, spinal cord, spinal ganglion, peripheral nerve, or target muscle is performed.
    a. In 80% of the experiments, the injection of the viral vector is performed simultaneously with the lesioning procedure resulting in only one

exposure of the animal to surgery *(max 1x).*
   b. In 20% of the experiments, the target expression needs to be present prior- or after the lesion has been applied resulting in a separate surgery (*max 1x*).
5) Dependent on the viral vector system or transgenic mouse used, administration of transgene-inducing agent or pharmaceuticals are needed continuously or alternating, as follows:
   a. Enteral: diet supplemented with doxycyclin (during certain periods of the experiment)
   b. Parenteral: intravenous, intramuscular, intraperitoneal injection of luciferine a substrate for luciferase a reporter gene used to monitor the activity of some vectors. Subsequent imaging to visualize viral vector mediated expression of the reporter gene luciferase is performed in the IVIS under isoflurane anaesthesia. These measurements generally take 5 minutes, but maximally 30 minutes (*max 1x/wk, max 24x*).
   c. Parenteral: I.P. injection of tamoxifen (*max 3x*).
6) *Testing the efficacy of the treatment by assessment of recovery of function:* The initial loss and gradual gain of function occurs over a period of maximal 12 months and will be evaluated by performing a subset of the function tests as described in (1). The frequency of testing is:
   a. Early following the lesion (first 4-8 weeks), (bi-) weekly tests need to be performed in order to evaluate the dynamics of this initial recovery phase.
   b. After 4-8 weeks, gain of function will start to level off and a weekly- to bimonthly testing frequency up to the final 12th month is sufficient.
7) *Testing the efficacy of the treatment by assessment of anatomical parameters*:
   a. *Retrograde tracing to histologically visualize treatment efficacy prior to sacrifice.* Administration of an antero- or retrograde tracer by:
      1. Surgically exposing the peripheral nerve followed by tracer application under adequate anesthesia and analgesia (*max. 1x*)
      2. Surgically exposing the skull/spinal cord followed by intracranial or intraspinal tracer injection under adequate anesthesia and analgesia (*max.1 x*)
   b. Perfusion fixation of animals at specific time points after the lesion by sacrificing the animals by administration of an overdose of barbiturate (I.P) followed by transcardial perfusion/fixation, or via CO2/O2 sedation followed by dissection of the relevant tissues for extensive histological analysis of the tissue response to the treatment.
8) (Optional) Withdrawal of blood samples without anesthesia in the mouse, or under adequate anaesthesia in the rat. (*max 5x*).
9) (Optional) Imaging by a light source (luminescence): the intravenous or intraperitoneal administration of luciferin under isoflurane anesthesia for a period of max 10 minutes (*max 2x week up over a period of maximally 12 months*).
10) The animals will be sacrificed by administration of an overdose of barbiturate (I.P) followed by transcardial perfusion/fixation, or via CO2/O2 sedation followed by decapitation.

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

Quantitative analysis: prior to performing an experiment we perform statistical analysis (a power analysis) to ensure that we use the minimum number of animals per group that will be statistically sound and biological relevant.
Qualitative analysis (most of our experiments): the number is based in literature and/or years of experience with similar type of experiments. Moreover, these types of experiments will be performed sequentially in order to ensure that we will use the minimum number of rats or mice per group that is informative resulting in scientifically sound conclusions.

B. The animals

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

Mice: genetically modified and wild type; The mice are obtained from our own breedings or from a commercial licensed breeder.
Mice (adult): 750.

Rat (adult): 900. The rats are obtained from a commercial licensed breeder

The estimate of the total number of animals is primarily based on our experience over the past 5 years. This number of experiment and therefore the number of animals is dependent upon the number of involved researchers which is strongly dependent on the available funding. However, in general, an estimate for the total number of rats and mice is as follows: Function studies contain an average of 60 rats (containing experimental groups, positive- negative- and biological controls). In average 3 of these studies are performed each year, resulting in a total of 900 rats (5 year x 3 studies x n=60). The same is true for mouse function studies, resulting in 900 mice total.

Before we start our experiments we will write an application to the IvD. In this application we will exactly describe (among others) which considerations, facts and results have led to the proposition of the experiments, which specific question(s) we are trying to answer with the proposed animal experiments and what the ultimate goal is for the proposed experiments. Moreover we will describe in full detail the experimental design, argue the number of animals in the experiments, describe (i.a.) human endpoints, alternatives, nature of discomfort. Experiments will only be started upon IvD approval.

C. Re-use

Will the animals be re-used?

☒ 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'?

☐ 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.

The procedures described in this project are based on a large body of scientific- and experimental experience in both rats and mice. Testing of the intervention-techniques will be performed *in-silico* and *in-vitro* as much as possible prior to performing an animal experiment. However, to fully understand and study the proposed mechanisms/targets, animal studies are necessary as the complexity of the processes occurring following a lesion can only be achieved in a complete organism.

These studies are conducted in both rat and mouse worldwide, making translation and extrapolation of data between research-groups more feasible. Experiments will be executed in succession and, if needed, small explorative studies will be performed to provide the necessary insight in variation and expected results. On basis of this previous work and experience, statistical analysis can be performed to determine the maximum number of animals needed to obtain valuable data.

In the case when transgenic mice are used; presence of an existing mouse line is checked, and/or an attempt is made to obtain the target transgenic mouse in as little breeding steps as possible, reducing breeding time and number of animals. In addition, mice with inducible alleles will be used when possible, resulting in a normal phenotype until induction.

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

All surgical procedures resulting in animal suffering or pain will be performed under adequate anaesthesia and analgesia. Close postoperative monitoring will be performed and humane endpoints applied. All available resources to reduce pain, fear or suffering will be employed.

Procedures will only be performed by competent personnel, as mandatory.
Adverse environmental effects are not present.
All rats and mice will be socially housed and provided with tools for environmental enrichment. For some periods during the experiments they are housed under strict DM2, DM1 regulations (no discomfort consequences).

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.

The proposed experiments are not carried out as a result of legal requirements.

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?

☒ 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?

☒ 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.

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

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

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

anaesthesia and analgesia is used.

I. Other aspects compromising the welfare of the animals

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

As a result of the applied loss of innervation/ neurodegeneration, autotomy, a response consisting of excessive licking and biting at the affected area, might occur. In time this can lead to tissue damage. In our experience this behavior occurs mainly between 2-8 weeks post lesion and is *not* present in all types of lesions. Spinal cord and dorsal root lesions are the types of lesions were this occurs most frequently.
It is expected that in 33% of the experiments 2-5% of all animals will be experiencing these adverse effects to different degrees.
All animals will be frequently monitored for possible side effects.
Animals exhibiting any unexpected phenotype will be killed within a day.

Explain why these effects may emerge.

The precise mechanism behind autotomy is still largely unknown. This behaviour only occurs in an denervated area/limb and is in the majority of cases transient.

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

Animals will be monitored daily and if possible treatment will be initiated (topically or systemic).

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.

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

Weight loss (>20% weight loss compared to pre-operative bodyweight), abnormal/unexpected behaviour and/or posture, general signs of illness and/or discomfort. Autotomy. The discomfort will never be more that moderate.

Indicate the likely incidence.

Expected 2-5 % within time frame of the experiment.

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').

Moderate 100%. In most of the cases this discomfort is transient (1-2 days).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

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

the rats or mice will be killed for histological analysis.

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.

☒ 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.zbo-ccd.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'.	NVWA80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
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: Testing of the quality of viral vector batches

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.

A primary aim of our research is to unravel new mechanisms that govern or hamper neuroregeneration or neuroplasticity. A key approach that we use to test the efficacy of a specific target molecule is viral vector-mediated gene transfer to neurons or glia cells. Although the viral vector technique has become more and more a standard technique, the generation of new viral vectors with potentially improved performance is an ongoing endeavor. For instance, of one of the most used viral vectors (adeno-associated viral vectors - AAV) an increasing number of variants ("serotypes") with specific cellular transduction profiles have

become available. Moreover we are currently developing vectors with regulatable transgene expression. Therefore it is necessary to have a protocol in place that allows testing the performance of newly generated vectors in small scale prior to their use in large animal experiments. A new vector batch that needs to be tested will only be tested in the tissue for which it is intended to be used later in the large experiment in which its efficacy is tested. Therefore this test protocol includes injections in the brain, spinal cord, peripheral nerve, and muscle without surgically inflicted damage.
Injection of a viral vector in the brain, spinal cord or the peripheral nerve or muscle will be performed by means of well-established (stereotactic) injection procedures. The typical primary outcome parameters that will be studied are: the transduction efficiency (number of cells transduced), the level of transgene expression per cell, the spread and cell type specificity obtained with a viral vector and, in the case of a regulatable viral vector, the inducibility and subsequent silencing of transgene expression.

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

This procedure can consist of the following steps:

1) Injection of a vector in the brain, spinal cord or the peripheral nerve or muscle. Dependent on the specific aim of the project for which the vector is generated the vector will be tested in the tissue of interest for that project (max. 1 x) For most vectors is will be sufficient to test the performance on a limited number of port-injection times, e.g. 2 weeks and 4 weeks. For other tests of viral vectors more elaborate testing will be required, e.g. for regulatable vectors where a gene is turned on and off.
2) Specific virus batch-dependent situations:
3) Dependent on the viral vector system or transgenic mouse used, administration of transgene-inducing agent or pharmaceuticals are needed continuously or alternating, as follows:
    a. Enteral: diet supplemented with doxycyclin (during certain periods of the experiment)
    b. Parenteral: intravenous, intramuscular, intraperitoneal injection of luciferine a substrate for luciferase a reporter gene used to monitor the activity of some vectors. Subsequent imaging to visualize viral vector mediated expression of the reporter gene luciferase is performed in the IVIS under isoflurane anaesthesia. These measurements generally take 5 minutes, but maximally 30 minutes (*max 1x/wk, max 24x*).
    c. Parenteral: I.P. injection of tamoxifen (*max 3x*).

    Vectors encoding the recombinase Cre will be tested in genetically modified mice that carry a floxed gene of interest or a floxed reporter gene which requires i.p injection of tamoxifen
4) At the end of the experiment the animals will be sacrificed by administration of an overdose of barbiturate (I.P) followed by transcardial perfusion/fixation, or via CO2/O2 sedation followed by decapitation. The expression of the transgene will be studied by histological analysis of the tissue or by biochemical analysis, e.g. and ELISA for GFP or GDNF.

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

We will not use statistical method to determine the number of animals in this particular procedure because the aim of this procedure is not to compare groups with different treatments. Based on previous experiments we know that an N=4 per group is normally sufficient to determine whether a viral vector batch works well or not. The N=4 is based on the following consideration: our injection techniques are well-established, however, occasionally we lose an animal because the injections does not go optimal. With an N=4 we always have at least 3 animals in which we will be able to investigate transgene expression.

B. The animals

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

Mice (adult): 180. Either commercial or wildtype/transgenic mice from our own breeding facility.
Rat (adult): 460. Commercially available.

The estimate of the total number of animals is primarily based on our experience over the past 5 years.
The number of experiments and therefore the number of animals is dependent upon the number of involved researchers which is strongly dependent on the available funding.
Adult mice. We estimate that a total number of 7 viral vector batches have to be tested in mice. We expect that 1 batch will have to be tested on 3 timepoints with 4 mice per time point. For the other 6 batches one time point would be suffcient, 4 mice per time point. Per year we need 36 mice. Total per 5 years: 180 mice. The justification of the use of mice is that we use viral vectors that express Cre, a recombinase, to knock-out a specific gene that is floxed in mice.The advantage of the use of Cre expressed via a viral vector is that this can be done in adult animals, that is in circumstances where the development of the animal has been completely normal.
Adult rats. We estimate that a total of 15 viral vector batches have to be tested in rats. Total rats per 5 years is 460. The justification of the use of rats is that our lesion and regeneration models are esthablished in rats.

C. Re-use

Will the animals be re-used?

☒ 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'?

☐ 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.

The aim of this protocol is to test the performance of viral vector batches prior to their use in larger animal experiments. This avoids that batches that do not perform as required are not used in larger animal experiments. This "pre-screening" of the performance of a viral vector batch results in the reduction of the use of animals because it avoids the use of a "bad" batch in larger experiments that would fail if the pre-screen would not have been done. Pre-screening is also a form of refinement.

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

All surgical procedures resulting in animal suffering or pain will be performed under adequate anaesthesia and analgesia. Close postoperative monitoring will

be performed and humane endpoints followed. All available resources to reduce pain, fear or suffering will be employed.
Procedures will only be performed by competent personnel, as mandatory.
Adverse environmental effects are not present.
All rats and mice will be socially housed with the appropriate environmental enrichment under standard and when required DM2, DM1 conditions.

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.

The proposed experiments are not carried out as a result of legal requirements.

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?

☒ 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?

☒ 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.

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

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

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

anaesthesia and analgesia is used

I. Other aspects compromising the welfare of the animals

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

Effects due to the performed surgery.
Effects as a consequence of the biological effects of the applied vector. No discomfort is expected.
All animals will be frequently monitored for possible side effects. Animals exhibiting any unexpected phenotype? will be killed within a day.

Explain why these effects may emerge.

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

Animals will be monitored daily and if possible treatment will be initiated (topically or systemic).

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.

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

Weight loss (>20% weight loss compared to pre-operative bodyweight), abnormal/unexpected behaviour and/or posture, general signs of illness and/or discomfort.

Indicate the likely incidence.

Expected 2-5 % within time frame of the experiment.

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').

Mild 100%

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

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

The rats or mice will be killed for histological analysis.

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.

☒ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
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1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	NVWA80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
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: # 5 Type of animal procedure: Monitoring and generation of novel genetically modified mice

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.

This procedure concerns the creation of genetically mice via DNA/RNA injection into oocyte, injection of genetically modified ES cells into blastocysts and/or via the CRISPR/Cas9 system. Moreover this procedure concerns the generation of crosses between mice with a floxed allel and Cre-expression mice lines in order to generate conditional null-mutant mice. As a consequence of this advanced breeding procedure mice may only have a gene deletion in a particular neuron or glia cell.

Welfare assessment of the novel mouse models will be performed according to the guidelines of the new EU directive. New transgenic lines and/or KO lines generated via classical methods and/or novel combinations of these aforementioned lines will be monitored for 2 generations to determine the absence or presence of mice with a deviant or hampered phenotype. Since whole body (compound) knock-outs will now be mostly replaced by cell specific knock-outs we expect that phenotyes will display considerable less adverse effects.

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

Generation of the mice according to classical methods:
1) Superovulation of donor mice.
   a) Administration of gonadotropin's (2 times) by subcutaneous or intraperitoneal injections followed by mating.
   b) Animals will be killed for the isolation of early (usually two or four cell stage) embryos.

2) Embryo recipients.
   a) Recipients for embryo transfer will be rendered pseudo pregnant by mating with a sterile (vasectomized) male.
   b) Genetically modified embryos will be implanted surgically or non-surgically into the reproductive tract.
   c) Embryo recipients, not as part of an experiment, will be killed after weaning of the pups at three weeks of age.

3) Weaned pups at 3 weeks of age: Tissue sampling for genotyping and/or identification via tail and earcut, respectively, under anesthesia (isoflurane).

Animals are killed by O2/CO2 method.

Welfare assessment:
Daily checks of the welfare of the mice on several common parameters (overall appearance, size, confirmation and growth, coat condition, behavior, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013

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

For these type of experiments statistical analysis is not performed since the purpose of the experiment is not to compare groups but to create viable novel mice lines for follow-up experiments. All techniques are state of the art and have been shown to be effective in generating GM mice with a smallest number of mice possible.

B. The animals

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

Mouse; Mus musculus: genetically modified and wild type adult mice. All mice are derived from the NIN, an establishment licensed by the NVWA, or from a registered commercial company.

For generation of GM mice we expect, based on our extensive experience, to generate max. 15 new lines over the next 5 years. For the creation of a new GM mouse lines we will use on average max. 150 mice (according to the besluit biotechnologie). Based on these numbers in total a maximum of 2250 mice will be required.

Welfare assesment: we expect to generate over the next 5 years 15 new GM lines for which we have to perform the welfare assesment. For 2 generations, 7 males and 7 females control and GM mice. We therfore need in total: 15 (new (compound) lines) * 2 (generation) * 28 ((7 male +7 female = 14 GM mice +

(7 male + 7 female = 14 control mice)) = 840 mice for the welfare assessment.

Taken together within the context of this procedure we need 2250 + 840 mice = 3090 mice

A large portion of the newly generated GG mice will be floxed mice, which have no phenotype by definition, and which are not part of the welfare assesment protocol. We will not breed new GM mice showing a hampered phenotype.

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

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

- (Vasectomized) males will also be used by the other groups of the NIN if required for their experiments, thereby reducing the number of (vasectomized) males used for the generation of GM mice.
  Mice used for welfare assessment, might be used for experiments described in procedures 3.4.4.1, 3.4.4.2 and 3.4.4.3.

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

☒ 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.

We start the generation of a new GM mouse line only after we are convinced that based on molecular screens in human tissue or animals (procedure 3.4.4.1), and in vitro experiments (procedure 3.4.4.2) the creation of a new line is essential for in vivo functional and mechanistic studies. Animal studies are essential unavoidable if we want to obtain comprehensive knowledge on the function of specific genes in processes of neuroregeneration and plasticity. The CRISPR/Cas9 system allows us, if required, to genetically modify multiple (that is up to 5 different) genes in a single experiments. This may strongly reduce the number of mice used for the generation and/or breeding of these GM mice.

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

All possibilities to reduce pain, fear or suffering will be used. There are no negative environmental effects; all mice will be housed under well-controlled DM1 conditions.

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.

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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.

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

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

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

I. Other aspects compromising the welfare of the animals

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

We do not expect to find additional adverse effect. This is the direct result of how we create our constructs for the generation of GM mice.

Explain why these effects may emerge.

We do not expect to find other adverse effect.

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

Daily monitoring of our mice; immediate action will be taken immediately if unexpectedly any adverse effect will become visible.

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.

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

Indicate the likely incidence.

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').

Vasectomized males: moderate
Donors: moderate 100%
Foster mothers: moderate 100%
GM mice: no to mild 100% (welfare assessment).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

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

The donor females will be killed as part of the experiments.
The foster females will be killed after the experiment (at the stage of weaning of the pups).

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.

☒ Yes

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD801002015104

Bijlagen
2

Datum 22-05-2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Geachte heer/mevrouw [redacted]
Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 22 mei 2015.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD801002015104. 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. Zodra uw aanvraag compleet is, ontvangt u binnen veertig werkdagen een beslissing op uw aanvraag. 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 wordt uw aanvraag buiten behandeling 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.zbo-ccd.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	80100
Naam instelling of organisatie:	Kon. Ned. Academie van Wetenschappen
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	54667089
Postbus:	19121
Postcode en plaats:	1100 GC AMSTERDAM
IBAN:	NLDEUT056900054
Tenaamstelling van het rekeningnummer:	[redacted]

Gegevens verantwoordelijke onderzoeker	
Naam:	[redacted]
Functie:	Group Leader
Afdeling:	Group Regeneration of Sensory Motor Systems
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	Biotechnicus
Afdeling:	Group Regeneration of Sensory Motor Systems
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

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

Over uw project

Geplande startdatum:	1 juli 2015
Geplande einddatum:	1 juli 2020
Titel project:	Developing strategies to promote repair or plasticity of the central and peripheral
Titel niet-technische samenvatting:	Ontwikkeling van strategieën om de regeneratie van zenuwweefsel te bevorderen
Naam DEC:	DEC KNAW
Postadres DEC:	[redacted] Amsterdam
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 741,-
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] DEC-advies [x] er zijn in totaal 5 appendixen

Ondertekening

Naam:	[redacted]
Functie:	Directeur Instituten KNAW
Plaats:	Amsterdam
Datum:	18 mei 2015

Centrale Commissie Dierproeven

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Aanvraagnummer
AVD801002015104

Bijlagen
2

Datum 22-05-2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 22 mei 2015
Vervaldatum: 21 juni 2015
Factuurnummer: 201570104

Omschrijving	**Bedrag**
Betaling leges projectvegrunning dierproeven Betreft aanvraag AVD801002015104	€ 741,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL28RBOS 056.99.96.066 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 20401, 2500 EK te 's Gravenhage.

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD801002015104

Datum
Betreft Vervolg Aanvraag projectvergunning Dierproeven

Geachte heer/mevrouw [redacted]
Op 22 mei 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project Developing strategies to promote repair or plasticity of the central and peripheral met aanvraagnummer AVD801002015104. Uw aanvraag wordt in behandeling genomen. In deze brief leest u wanneer u een beslissing kunt verwachten.

Wanneer een beslissing
Wij nemen uiterlijk 17 juni 2015 een beslissing. Als wij nog informatie nodig hebben, kan dit later worden. Voor een complexe aanvraag staat een langere termijn. In beide gevallen ontvangt u daarover bericht. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

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

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Kon. Ned. Academie van Wetenschappen
[redacted]
Postbus 19121
1100 GC AMSTERDAM

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

Onze referentie
Aanvraagnummer
AVD801002015104

Datum
Betreft Vervolg Aanvraag projectvergunning Dierproeven

Geachte heer/mevrouw [redacted]
Op 22 mei 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project Developing strategies to promote repair or plasticity of the central and peripheral met aanvraagnummer AVD801002015104.

DEC advies gevraagd
Uw aanvraag is naar DEC Kon. Ned. Academie van Wetenschappen gestuurd. Zij zal hierover advies aan de CCD uitbrengen. Als de DEC vragen heeft, zal zij contact met u opnemen.
Uw aanvraag wordt door een andere dan de door u aangegeven DEC van een advies voorzien. nvt

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

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Format DEC-advies

Maak bij de toepassing van dit format gebruik van de bijbehorende toelichting, waarin elke stap in het beoordelingsproces wordt toegelicht

A. Algemene gegevens over de procedure

1. Aanvraagnummer: AVD/801002015104
2. Titel van het project: Developing strategies to promote repair or plasticity of the central and peripheral nervous system.
3. Titel van de NTS: Ontwikkeling van strategieën om de regeneratie van zenuwweefsel te bevorderen.
4. Type aanvraag:
   - ✓ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer
5. Contactgegevens DEC:
   - naam DEC: KNAW
   - telefoonnummer contactpersoon: [redacted]
   - mailadres contactpersoon: [redacted]
6. Adviestraject (data dd-mm-jjjj):
   - ✓ ontvangen door DEC: 17-04-2015
   - ✓ aanvraag compleet: 30-04-2015
   - ✓ in vergadering besproken: 23-04-2015
   - ✓ anderszins behandeld: n.v.t.
   - ✓ termijnonderbreking(en): n.v.t.
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen:
   - ☐ aanpassing aanvraag:
   - ✓ advies aan CCD: 22-05-2015
7. Eventueel horen van aanvrager
   - Datum: n.v.t.
   - Plaats:
   - Aantal aanwezige DEC-leden:
   - Aanwezige (namens) aanvrager:
8. Correspondentie met de aanvrager:
   - Datum 24-04-2015
   - Strekking: completering van de aanvraag
   - Datum antwoord 20-05-2015
   - Strekking van de antwoorden: de aanvraag is gecompleteerd
9. Eventuele adviezen door experts (niet lid van de DEC): geen

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig. Het omvat dierproeven in de zin der wet.

2. De aanvraag betreft een nieuwe aanvraag. Er is enige overlap met een aantal al van een positief advies voorziene DEC-protocollen.
3. De DEC is competent om over deze projectvergunningsaanvraag te adviseren. De benodigde expertise op dit wetenschappelijk terrein is aanwezig binnen de DEC. Geen van de DEC-leden is betrokken bij het betreffende project.
4. Vanwege betrokkenheid bij het betreffende project is een aantal DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, niet betrokken bij de advisering: n.v.t.

C. Beoordeling (inhoud):

1. Het project is wetenschappelijk verantwoord.

2. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.

3. De doelstelling, in relatie tot de uitvoering, is helder omschreven; te weten het verkrijgen van fundamenteel wetenschappelijke inzichten in 1) de biologische mechanismen van het afsterven van zenuwcellen en de biologische mechanismen van zenuwweefselregeneratie na het optreden van schade en 2) de toepasbaarheid van die kennis in de ontwikkeling van therapeutische strategieën om het verlies van zenuwcellen te voorkomen of om de uitgroei van beschadigde zenuwuitlopers te stimuleren. Op termijn kunnen de resultaten leiden tot nieuwe behandelingsmethoden voor de humane patiënt met zenuwschade.

   Het fundamenteel wetenschappelijke belang acht de DEC substantieel. Het verkrijgen van wetenschappelijke kennis van de processen en factoren die ten grondslag liggen aan regeneratie van zenuwweefsel (waaronder het herstel van zenuwceluitlopers en het voorkomen van de celdood van zenuwcellen) is essentieel voor het ontwikkelen van nieuwe therapeutische strategieën. Inzichten in het gebruik van gentherapie met behulp van virale vectoren om de geïdentificeerde factoren te kunnen inbrengen op de juiste plaats in het geval van zenuwschade is naar de mening van de DEC van substantieel belang. Het project dient een belangrijk maatschappelijk belang, gezien de grote groep patiënten met zenuwweefselschade.

4. De gekozen strategie en experimentele aanpak in combinatie met de infrastructuur op het Nederlands Herseninstituut en de expertise van de betrokken onderzoeksgroep bieden een realistisch uitzicht op het behalen van de beoogde doelstellingen binnen gevraagde looptijd van het project. Het project bouwt voort op een langlopende lijn van onderzoek van een grote groep onderzoekers. Over de afgelopen jaren zijn met een vergelijkbare strategie en aanpak belangrijke wetenschappelijk resultaten behaald, resulterend in vele publicaties in vooraanstaande tijdschriften. Het onderzoek wordt financieel gesteund door verschillende onafhankelijke subsidiegevers. Er zijn internationale samenwerkingsverbanden en er zijn sterke relaties met de kliniek die een

vertaling van de bevindingen van het onderzoek naar de kliniek zullen vergemakkelijken.

5. Alle dieren worden gefokt voor het gebruik in dierproeven, er is geen sprake van afwijkende huisvesting en/of hergebruik. Er is geen sprake van bedreigde diersoorten, niet-menselijke primaten, zwerfdieren en/of dieren in/uit het wild. De toegepaste methoden voor anesthesie/euthanasie zijn conform de Richtlijn.

6. Het cumulatieve ongerief gepaard gaand met de dierproeven, zoals beschreven in de vijf verschillende type dierproeven, is naar inschatting van de DEC licht (Type dierproef 2, 4 en 5) of matig (Type dierproef 1 en 3) ongerief. In het merendeel van de gevallen met matig ongerief is de duur van het ongerief beperkt tot 1-2 dagen en is er een beperkt risico op onbedoelde bijwerkingen in de vorm van autotomie. Deze inschatting van de DEC is in overeenstemming met het niveau van cumulatief ongerief zoals dat is geclassificeerd door de onderzoekers. Dit is gebaseerd op hun ruime ervaring met de gebruikte modellen in vergelijkbare dierproeven.

7. Binnen het project wordt maximaal gebruik gemaakt van methoden die de voorgestelde dierproeven geheel of gedeeltelijk **vervangen.** Een belangrijk onderdeel van de experimentele strategie is de gefaseerde opzet. In de eerste fase, voorafgaand aan de dierproeven, vindt een uitgebreide screening plaats met weefsel afkomstig van patiënten en van de Nederlandse Hersenbank. Na deze fase zijn er go/no-go-beslissingsmomenten, voordat tot het uitvoeren van bioassays wordt besloten.
Nieuwe inzichten in de processen die zenuwweefselherstel reguleren kunnen op dit moment alleen maar verkregen worden in een intact organisme. Deze processen, waarbij verschillende typen cellen betrokken zijn binnen een gecompliceerde anatomische context, zijn zeer complex en kunnen niet met cellijnen worden bestudeerd. Naar het oordeel van de DEC zijn er geen alternatieven beschikbaar voor het voorgestelde gebruik van intacte dieren om te doelstelling van dit project te realiseren.

8. In het project wordt optimaal tegemoet gekomen aan de vereisten van **vermindering** van dierproeven. De onderzoeksgroep heeft een jarenlange ervaring opgebouwd met dit soort experimenten en door een veelal gefaseerde opzet wordt per experiment niet meer dan het minimaal benodigde aantal dieren ingezet. Voorafgaand aan de kwantitatieve experimenten wordt op basis van literatuurgegevens, eigen historische data of een specifiek hiertoe uitgevoerd pilot experiment de groepsgrootte bepaald. Technieken en procedures worden zorgvuldig toegepast. Het aantal te gebruiken dieren is realistisch geschat.
Voor de bioassays zijn op beperkte schaal proefdieren nodig (met licht ongerief) maar de uitkomsten van deze proeven leiden tot de selectie van factoren met een duidelijk effect en hiermee wordt het aantal dierproeven met

zenuwschademodellen (met matig ongerief) verminderd en wordt de kans op het verkrijgen wetenschappelijk relevante resultaten verhoogd.
Voor het genereren van genetisch gemodificeerde diermodellen waarin de gentherapie experimenten worden gedaan zijn naar verhouding veel dieren nodig. Ook ontstaat daarbij onvermijdelijk een overschot van dieren die wel gefokt worden, maar niet worden gebruikt in het onderzoek. De commissie acht dat aanvaardbaar in het licht van het feit dat de gentherapie experimenten, zowel in methodologisch opzicht (onderzoek naar de functie van een gen in beschadigd zenuwweefsel), als mogelijke toekomstige klinische behandeling, van essentieel belang zijn voor dit project.

9. De uitvoering van het project is in overeenstemming met de vereisten van **verfijning** van dierproeven en is zo opgezet dat de dierproeven met zo min mogelijk ongerief worden uitgevoerd.
Bij de opzet wordt rekening gehouden met dierenwelzijn en wel op de volgende manieren: 1) het gebruik van adequate anesthesie en analgesie waar nodig, 2) een intensieve monitoring van de proefdieren na de inductie van zenuwweefselschade, 3) het gebruik van weefselspecifieke genetisch gemodificeerde muizen, 4) een monitoring op het optreden van onverwacht constitutioneel ongerief van nieuwe gecreëerde genotypes.
Er is geen sprake van belangwekkende milieueffecten.

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

D. Ethische afweging

De centrale vraag voor de ethische afweging is of het belang van het doel van dit project opweegt tegen het ongerief dat de dieren ondergaan (geclassificeerd als licht of matig).Het doel van het project is het verkrijgen van fundamenteel wetenschappelijke inzichten in: 1) de biologische oorzaken van het afsterven van zenuwcellen en het (vrijwel volledig) ontbreken van zenuwweefselregeneratie en 2) de toepasbaarheid van die kennis in de ontwikkeling van een strategie (in het bijzonder toepassing van virale gentherapie) om het verlies van zenuwcellen te voorkomen of om de uitgroei van beschadigde zenuwuitlopers te stimuleren.
Het onderzoek is primair fundamenteel wetenschappelijk van karakter. De verwachting is dat de resultaten op den duur kunnen bijdragen aan nieuwe therapieën om de gevolgen van zenuwweefselschade voor patiënten te verminderen. Voor een grote groep patiënten met diverse typen zenuwweefselschade van is het van aanzienlijk belang dat er uitzicht ontstaat op nieuwe therapieën die de kwaliteit van hun leven aanmerkelijk zal kunnen verhogen.

Het fundamenteel wetenschappelijke onderzoek in dit project is van aangetoonde en excellente kwaliteit. De onderzoeksgroep beschikt over ruime ervaring met de gekozen onderzoeksstrategie en met de vijf beschreven type dierproeven.

De classificatie van het ongerief van de dieren in de verschillende typen dierproeven is licht of matig. De intrinsieke waarde van het dier wordt door de laesiemodellen in lichte mate aangetast wanneer de toegebrachte schade resulteert in een lichte verlamming. Bij de 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. De DEC onderschrijft dat de doelstellingen niet zonder het gebruik van proefdieren kunnen worden behaald.

De DEC is van mening dat de resultaten van dierproeven zullen bijdragen aan het behalen van het geformuleerde doel en schat de kans op het realiseren van de fundamenteel wetenschappelijke doelstellingen in als hoog. Het project is uit wetenschappelijk oogpunt verantwoord. De verkregen fundamenteel wetenschappelijke kennis is essentieel om te komen tot een toepassing van regeneratieve (gen)therapieën in patiënten met zenuwweefselschade. De verwachting is dat deze nieuwe therapieën effectiever zullen zijn dan de huidige therapieën. Het gaat om een grote groep patiënten met uiteenlopende, op dit moment nog slecht behandelbare, aandoeningen. Het maatschappelijk belang is daarom groot.
De DEC komt tot de conclusie dat de doeleinden van het project het voorgestelde gebruik van de proefdieren en het daarmee samenhangende ongerief van de proefdieren rechtvaardigt.

E. Advies

1. Advies aan de CCD
   - ✓ **De DEC adviseert de vergunning te verlenen**
2. Het uitgebrachte advies is gebaseerd op consensus.
3. Er zijn geen knelpunten of dilemma’s gesignaleerd tijdens het beoordelen van de aanvraag of het formuleren van het advies.

[redacted]

[redacted]

Aan de CCD
t.a.v. [redacted]
Postbus 20401
2500 EK Den Haag

Amsterdam, 12 juni, 2015

Geachte mevrouw [redacted]

Hierbij beantwoord ik uw vragen betreffende het project “Developing strategies to promote repair or plasticity of the central and peripheral nervous system” met nummer AVD 801002015104 zoals gesteld in uw e-mails van 5 juni en 11 juni j.l.

Uw vraag (5 juni): In de beschrijving dierproeven 3.4.4.3; Injury models: Bij punt B beschrijft u in de eerste alinea een aantal van 750 genetisch gemodificeerde of WT muizen. Verderop in de tekst berekend u voor de injury models 900 ratten en 900 muizen. Het wordt uit de tekst niet helemaal helder of het totale aantal 750 GGO muizen + 900 ratten + 900 muizen is. Kunt u dit toelichten en als nodig in de tekst aanpassen?

Mijn antwoord: er zijn voor dit type dierproef in totaal 900 muizen en 900 ratten nodig. De berekening van het aantal muizen op 750 berust op een (reken)fout. In de tekst van 3.4.4.3 is voor beide diersoorten nu 900 aangehouden. In de NTS stond het juiste aantal dieren aangegeven. Bijgevoegd is een tabel met de dieren aantallen per appendix uitgesplitst naar diersoort en verwacht ongerief (Bijlage Table of Groups). Wij verzoeken u deze tabel als bijlage bij de PVA op te nemen in het dossier.

Uw vraag (5 juni): In beschrijving dierproeven 3.4.4.4; testing of the quality of viral vector batches, zit een tekstuele inconsequentie: bij punt A beschrijft u dat de handelingen “without surgically inflicted damage” plaatsvinden . Bij punt D refereert u aan “all surgical procedures” . Kunt u bevestigen dat deze handelingen zonder chirurgie plaatsvinden? Mocht er wel chirurgie noodzakelijk zijn dan zou dit consequenties voor de ongerief inschatting “licht” kunnen hebben, kunt u dit toelichten?

Mijn antwoord: “all surgical procedures” onder punt D refereert naar de procedures die nodig zijn om de vector te injecteren en refereert niet naar het maken van een laesie. Het maken van een laesie is in dit type dierproef niet aan de orde. Dit is nu verwoord in de tekst van 3.4.4.4 onder D waar is toegevoegd: ….REQUIRED FOR VECTOR INJECTION….

Uw vraag (11 juni): U beschrijft het gebruik van ratten en muizen, de CCD hecht eraan het aantal in voorraad gedode dieren terug te dringen en heeft daarom de vraag of u in zijn algemeenheid kunt toelichten of u (behalve voor de fok); voor de experimentele procedures beide geslachten kunt gebruiken of in het geval u 1 geslacht gebruikt motiveren waarom dit is?

[redacted]

[redacted]

Mijn antwoord: we streven ernaar transgene lijnen te genereren en in stand te houden met een minimaal fokoverschot en gebruiken hiervoor zowel mannen als vrouwen. De experimentele procedures met *muizen* beschreven in Appendices 1 t/m 4 worden uitgevoerd op zowel vrouwen als mannen. Voor *ratten* geldt dat we deze aanschaffen bij een commerciële fokker. Vrijwel al onze experimentele procedures met ratten doen we met vrouwen omdat we willen uitsluiten dat er extra variatie ontstaat t.g.v. eventuele geslachtsverschillen.

Uw vraag (11 juni): In Bijlage 3.4.4.5. beschrijving dierproeven is punt H. niet volledig ingevuld, wij interpreteren dit als dat er NO in plaats van YES aangekruist had moeten worden.

Mijn antwoord: De muizen in Appendix 5 opgevoerd voor ongeriefmonitoring (n=840) zullen naar onze inschatting geen pijn ondervinden (licht ongerief) . Echter de muizen benodigd voor het genereren van TG lijnen (n=2250; gevasectomeerde mannen, donoren, fosters) zullen wel pijn ondervinden (matig ongerief). Zie ook de bijlage “Bijlage Table of Groups”. Dit is de reden waarom we YES hebben aangekruist. De vraag is hoe binnen de kaders van het formulier duidelijk te maken dat er ook dieren zijn waarvoor NO van toepassing is. Ons voorstel is om zowel NO als YES aan te kruisen met een korte toelichting onder het YES met de volgende tekst: “Animals involved in welfare assessment (n = 840) will experience no pain. Animals involved in the generation of TG lines (n=2250) will experience pain as a consequence of the procedures and they receive adequate anaesthesia and analgesia.”

Hopende u hiermee voldoende te hebben geïnformeerd.

Met vriendelijke groet,

[redacted]

[redacted]

Bijlagen:
Appendices 3, 4, 5 met correcties
AVD801002015104 - Bijlage Table of Groups

cc. DEC-KNAW

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.zbo-ccd.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'.	NVWA 80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
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: Injury models (functional and histological analysis): Injection of cells or viral vectors in lesioned animals.

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.

Our aim is to determine whether our identified target molecules and intervention methods (i.e. viral vector or cell-mediated gene delivery of a target, by using transgenic mice with overexpression or knock-down of a target) will improve both anatomical and functional recovery following a lesion to the brain, spinal cord, or peripheral nerve. Therefore, following a lesion we will perform several non-invasive function tests and/or an electrophysiological test on each

animal at several time points after induction of the lesion and the therapeutic intervention. This will provide insight in the full spectrum of the induced functional deficit and the degree of subsequent recovery of function per animal in time.
The brain, spinal cord or peripheral nerve lesions will be made by means of well-established surgical procedures. These lesion types are chosen because they represent clinically relevant injuries in which we can test the efficacy of a specific intervention. In different parts of the CNS different processes underlie degeneration and regeneration and it is therefore important to study different parts of the CNS (brain, spinal cord, spinal ganglion, peripheral nerve) and innervated target muscles.
The cells or viral vectors will be injected via small needles in the area of study.
Activation or inhibition of transgenes encoded in the viral vectors or transgene mouse via pharmaceuticals (e.g. doxycycline, tamoxifen) might be necessary to regulate transgene expression in time.
Intravenous application of a substrate (e.g. luciferine) might be necessary to perform an imaging study and visualize viral vector mediated expression of the reporter gene luciferase.
Assessment of recovery of function is obtained by performing multiple function tests at multiple time points.
Animals might be injected under anaesthesia with an antero/retrograde tracer prior to killing the animals to allow histological assessment of regeneration/sprouting process at the intermediate or the final stages of recovery.
At the end of the experiments in all cases the animals will be killed and tissues will be harvested for further analysis, allowing direct correlation between the individual degree of function recovery and histological parameters. These tissues can be subjected to: histological sectioning followed by immunohistochemical- or in-situ hybridisation staining or biochemical analysis (ELISA, FACS, Microarray, RNA/ DNA/ Protein extraction).

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

This procedure will consist of the following steps:

1) *Pre-training and baseline measurement of the animals using behavioural function tests*. Acclimatization of the animals to both the handling and testing-environment will result in reliable baseline data with little variation. This step involves:
    a. Pre-training on our non-invasive function tests performed on freely moving animals to obtain baseline values *(max: 1x exposure/ test/ day, for 2 weeks).* In general, animals performing one or more non-invasive function tests including, for example: 1) cross a beam, rope or narrow corridor, walking from platform to platform without being forced. 2) Grip a horizontal bar and pull after which the maximum applied force is measured. 3) Walk in an open field or cylinder while being observed by the researcher who is scoring naturally behaviour 'events'. Dependent on the aim of the experiment the relevant test(s) and variants thereof will be selected and applied.
2) Baseline measurement of electrophysiological (CMAP) measurement under adequate anaesthesia. *(max: 2x)*
3) *Application of a lesion to the brain, spinal cord or peripheral nerve*. Under adequate anaesthesia and postoperative analgesia, the skull, spinal cord or peripheral nerve is exposed and a unilateral or bilateral lesion is made by means of damaging of a nerve tract.
    a. *Brain*: injection of a toxin systemically (I.P.) or intracranially, or by means of mechanical injury with microscissors *(max 1x).*
    b. *Spinal cord*: At cervical or thoracic or lumbar level, access to the spinal cord is gained and a uni- or bilateral lesion of the rubrospinal tract, corticospinal tract, dorsal column or dorsolateral columns is performed by means of microscissors *(max 1x).*
    c. *Peripheral nerve*: After gaining access to the peripheral nerve lesion site, unilateral crush or transection or spinal root avulsion is performed. *(max 1x).*
4) *Viral vector or cell-mediated gene delivery of a target.* Under adequate anaesthesia and postoperative analgesia a (stereotaxic) injection of a viral vector (an control vector encoding GFP or 'repair experiments' with an experimental vector encoding a target gene) or of cells (control cells expressing GFP or cells expressing an experimental target gene), or of tissue grafts (virally transduced or not), in a specific brain area, spinal cord, spinal ganglion, peripheral nerve, or target muscle is performed.
    a. In 80% of the experiments, the injection of the viral vector is performed simultaneously with the lesioning procedure resulting in only one

exposure of the animal to surgery *(max 1x).*
   b. In 20% of the experiments, the target expression needs to be present prior- or after the lesion has been applied resulting in a separate surgery (*max 1x*).
5) Dependent on the viral vector system or transgenic mouse used, administration of transgene-inducing agent or pharmaceuticals are needed continuously or alternating, as follows:
   a. Enteral: diet supplemented with doxycyclin (during certain periods of the experiment)
   b. Parenteral: intravenous, intramuscular, intraperitoneal injection of luciferine a substrate for luciferase a reporter gene used to monitor the activity of some vectors. Subsequent imaging to visualize viral vector mediated expression of the reporter gene luciferase is performed in the IVIS under isoflurane anaesthesia. These measurements generally take 5 minutes, but maximally 30 minutes (*max 1x/wk, max 24x*).
   c. Parenteral: I.P. injection of tamoxifen (*max 3x*).
6) *Testing the efficacy of the treatment by assessment of recovery of function:* The initial loss and gradual gain of function occurs over a period of maximal 12 months and will be evaluated by performing a subset of the function tests as described in (1). The frequency of testing is:
   a. Early following the lesion (first 4-8 weeks), (bi-) weekly tests need to be performed in order to evaluate the dynamics of this initial recovery phase.
   b. After 4-8 weeks, gain of function will start to level off and a weekly- to bimonthly testing frequency up to the final 12th month is sufficient.
7) *Testing the efficacy of the treatment by assessment of anatomical parameters*:
   a. *Retrograde tracing to histologically visualize treatment efficacy prior to sacrifice.* Administration of an antero- or retrograde tracer by:
      1. Surgically exposing the peripheral nerve followed by tracer application under adequate anesthesia and analgesia (*max. 1x*)
      2. Surgically exposing the skull/spinal cord followed by intracranial or intraspinal tracer injection under adequate anesthesia and analgesia (*max.1 x*)
   b. Perfusion fixation of animals at specific time points after the lesion by sacrificing the animals by administration of an overdose of barbiturate (I.P) followed by transcardial perfusion/fixation, or via CO2/O2 sedation followed by dissection of the relevant tissues for extensive histological analysis of the tissue response to the treatment.
8) (Optional) Withdrawal of blood samples without anesthesia in the mouse, or under adequate anaesthesia in the rat. (*max 5x*).
9) (Optional) Imaging by a light source (luminescence): the intravenous or intraperitoneal administration of luciferin under isoflurane anesthesia for a period of max 10 minutes (*max 2x week up over a period of maximally 12 months*).
10) The animals will be sacrificed by administration of an overdose of barbiturate (I.P) followed by transcardial perfusion/fixation, or via CO2/O2 sedation followed by decapitation.

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

Quantitative analysis: prior to performing an experiment we perform statistical analysis (a power analysis) to ensure that we use the minimum number of animals per group that will be statistically sound and biological relevant.
Qualitative analysis (most of our experiments): the number is based in literature and/or years of experience with similar type of experiments. Moreover, these types of experiments will be performed sequentially in order to ensure that we will use the minimum number of rats or mice per group that is informative resulting in scientifically sound conclusions.

B. The animals

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

Mice: genetically modified and wild type; The mice are obtained from our own breedings or from a commercial licensed breeder.

Mice (adult): 900.
Rat (adult): 900. The rats are obtained from a commercial licensed breeder
The estimate of the total number of animals is primarily based on our experience over the past 5 years. This number of experiments and therefore the number of animals is dependent upon the number of involved researchers which is strongly dependent on the available funding. However, in general, an estimate for the total number of rats and mice is as follows: Function studies contain an average of 60 rats (containing experimental groups, positive- negative- and biological controls). In average 3 of these studies are performed each year, resulting in a total of 900 rats (5 year x 3 studies x n=60). The same is true for mouse function studies, resulting in 900 mice total.
Before we start our experiments we will write an application to the IvD. In this application we will exactly describe (among others) which considerations, facts and results have led to the proposition of the experiments, which specific question(s) we are trying to answer with the proposed animal experiments and what the ultimate goal is for the proposed experiments. Moreover we will describe in full detail the experimental design, argue the number of animals in the experiments, describe (i.a.) human endpoints, alternatives, nature of discomfort. Experiments will only be started upon IvD approval.

C. Re-use

Will the animals be re-used?

☒ 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'?

☐ 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.

The procedures described in this project are based on a large body of scientific- and experimental experience in both rats and mice. Testing of the intervention-techniques will be performed *in-silico* and *in-vitro* as much as possible prior to performing an animal experiment. However, to fully understand and study the proposed mechanisms/targets, animal studies are necessary as the complexity of the processes occurring following a lesion can only be achieved in a complete organism.
These studies are conducted in both rat and mouse worldwide, making translation and extrapolation of data between research-groups more feasible. Experiments will be executed in succession and, if needed, small explorative studies will be performed to provide the necessary insight in variation and expected results. On basis of this previous work and experience, statistical analysis can be performed to determine the maximum number of animals needed to obtain valuable data.
In the case when transgenic mice are used; presence of an existing mouse line is checked, and/or an attempt is made to obtain the target transgenic mouse in as little breeding steps as possible, reducing breeding time and number of animals. In addition, mice with inducible alleles will be used when possible, resulting in a normal phenotype until induction.

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

All surgical procedures resulting in animal suffering or pain will be performed under adequate anaesthesia and analgesia. Close postoperative monitoring will be performed and humane endpoints applied. All available resources to reduce pain, fear or suffering will be employed.
Procedures will only be performed by competent personnel, as mandatory.
Adverse environmental effects are not present.
All rats and mice will be socially housed and provided with tools for environmental enrichment. For some periods during the experiments they are housed under strict DM2, DM1 regulations (no discomfort consequences).

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.

The proposed experiments are not carried out as a result of legal requirements.

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?

☒ 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?

☒ 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.

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

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

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

anaesthesia and analgesia is used.

I. Other aspects compromising the welfare of the animals

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

As a result of the applied loss of innervation/ neurodegeneration, autotomy, a response consisting of excessive licking and biting at the affected area, might occur. In time this can lead to tissue damage. In our experience this behavior occurs mainly between 2-8 weeks post lesion and is *not* present in all types of lesions. Spinal cord and dorsal root lesions are the types of lesions were this occurs most frequently.
It is expected that in 33% of the experiments 2-5% of all animals will be experiencing these adverse effects to different degrees.
All animals will be frequently monitored for possible side effects.
Animals exhibiting any unexpected phenotype will be killed within a day.

Explain why these effects may emerge.

The precise mechanism behind autotomy is still largely unknown. This behaviour only occurs in an denervated area/limb and is in the majority of cases transient.

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

Animals will be monitored daily and if possible treatment will be initiated (topically or systemic).

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.

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

Weight loss (>20% weight loss compared to pre-operative bodyweight), abnormal/unexpected behaviour and/or posture, general signs of illness and/or discomfort. Autotomy. The discomfort will never be more that moderate.

Indicate the likely incidence.

Expected 2-5 % within time frame of the experiment.

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').

Moderate 100%. In most of the cases this discomfort is transient (1-2 days).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: ‘signatures’.

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

the rats or mice will be killed for histological analysis.

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.

☒ 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.zbo-ccd.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'.	NVWA80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
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: Testing of the quality of viral vector batches

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.

A primary aim of our research is to unravel new mechanisms that govern or hamper neuroregeneration or neuroplasticity. A key approach that we use to test the efficacy of a specific target molecule is viral vector-mediated gene transfer to neurons or glia cells. Although the viral vector technique has become more and more a standard technique, the generation of new viral vectors with potentially improved performance is an ongoing endeavor. For instance, of one of the most used viral vectors (adeno-associated viral vectors - AAV) an increasing number of variants ("serotypes") with specific cellular transduction profiles have

become available. Moreover we are currently developing vectors with regulatable transgene expression. Therefore it is necessary to have a protocol in place that allows testing the performance of newly generated vectors in small scale prior to their use in large animal experiments. A new vector batch that needs to be tested will only be tested in the tissue for which it is intended to be used later in the large experiment in which its efficacy is tested. Therefore this test protocol includes injections in the brain, spinal cord, peripheral nerve, and muscle without surgically inflicted damage.
Injection of a viral vector in the brain, spinal cord or the peripheral nerve or muscle will be performed by means of well-established (stereotactic) injection procedures. The typical primary outcome parameters that will be studied are: the transduction efficiency (number of cells transduced), the level of transgene expression per cell, the spread and cell type specificity obtained with a viral vector and, in the case of a regulatable viral vector, the inducibility and subsequent silencing of transgene expression.

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

This procedure can consist of the following steps:

1) Injection of a vector in the brain, spinal cord or the peripheral nerve or muscle. Dependent on the specific aim of the project for which the vector is generated the vector will be tested in the tissue of interest for that project (max. 1 x) For most vectors is will be sufficient to test the performance on a limited number of port-injection times, e.g. 2 weeks and 4 weeks. For other tests of viral vectors more elaborate testing will be required, e.g. for regulatable vectors where a gene is turned on and off.
2) Specific virus batch-dependent situations:
3) Dependent on the viral vector system or transgenic mouse used, administration of transgene-inducing agent or pharmaceuticals are needed continuously or alternating, as follows:
    a. Enteral: diet supplemented with doxycyclin (during certain periods of the experiment)
    b. Parenteral: intravenous, intramuscular, intraperitoneal injection of luciferine a substrate for luciferase a reporter gene used to monitor the activity of some vectors. Subsequent imaging to visualize viral vector mediated expression of the reporter gene luciferase is performed in the IVIS under isoflurane anaesthesia. These measurements generally take 5 minutes, but maximally 30 minutes (*max 1x/wk, max 24x*).
    c. Parenteral: I.P. injection of tamoxifen (*max 3x*).

    Vectors encoding the recombinase Cre will be tested in genetically modified mice that carry a floxed gene of interest or a floxed reporter gene which requires i.p injection of tamoxifen
4) At the end of the experiment the animals will be sacrificed by administration of an overdose of barbiturate (I.P) followed by transcardial perfusion/fixation, or via CO2/O2 sedation followed by decapitation. The expression of the transgene will be studied by histological analysis of the tissue or by biochemical analysis, e.g. and ELISA for GFP or GDNF.

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

We will not use statistical method to determine the number of animals in this particular procedure because the aim of this procedure is not to compare groups with different treatments. Based on previous experiments we know that an N=4 per group is normally sufficient to determine whether a viral vector batch works well or not. The N=4 is based on the following consideration: our injection techniques are well-established, however, occasionally we lose an animal because the injections does not go optimal. With an N=4 we always have at least 3 animals in which we will be able to investigate transgene expression.

B. The animals

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

Mice (adult): 180. Either commercial or wildtype/transgenic mice from our own breeding facility.
Rat (adult): 460. Commercially available.
The estimate of the total number of animals is primarily based on our experience over the past 5 years.
The number of experiments and therefore the number of animals is dependent upon the number of involved researchers which is strongly dependent on the available funding.
Adult mice. We estimate that a total number of 7 viral vector batches have to be tested in mice. We expect that 1 batch will have to be tested on 3 time points with 4 mice per time point. For the other 6 batches one time point would be suffcient, 4 mice per time point. Per year we need 36 mice. Total per 5 years: 180 mice. The justification of the use of mice is that we use viral vectors that express Cre, a recombinase, to knock-out a specific gene that is floxed in mice.The advantage of the use of Cre expressed via a viral vector is that this can be done in adult animals, that is in circumstances where the development of the animal has been completely normal.
Adult rats. We estimate that a total of 15 viral vector batches have to be tested in rats. Total rats per 5 years is 460. The justification of the use of rats is that our lesion and regeneration models are esthablished in rats.

C. Re-use

Will the animals be re-used?

☒ 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'?

☐ 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.

The aim of this protocol is to test the performance of viral vector batches prior to their use in larger animal experiments. This avoids that batches that do not perform as required are not used in larger animal experiments. This "pre-screening" of the performance of a viral vector batch results in the reduction of the use of animals because it avoids the use of a "bad" batch in larger experiments that would fail if the pre-screen would not have been done. Pre-screening is also a form of refinement.

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

All surgical procedures REQUIRED FOR VECTOR INJECTION resulting in animal suffering or pain will be performed under adequate anaesthesia and analgesia. Close postoperative monitoring will be performed and humane endpoints followed. All available resources to reduce pain, fear or suffering will be employed.

Procedures will only be performed by competent personnel, as mandatory.
Adverse environmental effects are not present.
All rats and mice will be socially housed with the appropriate environmental enrichment under standard and when required DM2, DM1 conditions.

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.

The proposed experiments are not carried out as a result of legal requirements.

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?

☒ 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?

☒ 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.

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

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

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

anaesthesia and analgesia is used

I. Other aspects compromising the welfare of the animals

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

Effects due to the performed surgery.
Effects as a consequence of the biological effects of the applied vector. No discomfort is expected.
All animals will be frequently monitored for possible side effects. Animals exhibiting any unexpected phenotype? will be killed within a day.

Explain why these effects may emerge.

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

Animals will be monitored daily and if possible treatment will be initiated (topically or systemic).

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.

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

Weight loss (>20% weight loss compared to pre-operative bodyweight), abnormal/unexpected behaviour and/or posture, general signs of illness and/or discomfort.

Indicate the likely incidence.

Expected 2-5 % within time frame of the experiment.

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').

Mild 100%

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

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

The rats or mice will be killed for histological analysis.

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.

☒ 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.zbo-ccd.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'.	NVWA80101
1.2	Provide the name of the licenced establishment.	Netherlands Institute for Neuroscience
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: # 5 Type of animal procedure: Monitoring and generation of novel genetically modified mice

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.

This procedure concerns the creation of genetically mice via DNA/RNA injection into oocyte, injection of genetically modified ES cells into blastocysts and/or via the CRISPR/Cas9 system. Moreover this procedure concerns the generation of crosses between mice with a floxed allel and Cre-expression mice lines in order to generate conditional null-mutant mice. As a consequence of this advanced breeding procedure mice may only have a gene deletion in a particular neuron or glia cell.

Welfare assessment of the novel mouse models will be performed according to the guidelines of the new EU directive. New transgenic lines and/or KO lines generated via classical methods and/or novel combinations of these aforementioned lines will be monitored for 2 generations to determine the absence or presence of mice with a deviant or hampered phenotype. Since whole body (compound) knock-outs will now be mostly replaced by cell specific knock-outs we expect that phenotyes will display considerable less adverse effects.

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

Generation of the mice according to classical methods:
1) Superovulation of donor mice.
   a) Administration of gonadotropin's (2 times) by subcutaneous or intraperitoneal injections followed by mating.
   b) Animals will be killed for the isolation of early (usually two or four cell stage) embryos.

2) Embryo recipients.
   a) Recipients for embryo transfer will be rendered pseudo pregnant by mating with a sterile (vasectomized) male.
   b) Genetically modified embryos will be implanted surgically or non-surgically into the reproductive tract.
   c) Embryo recipients, not as part of an experiment, will be killed after weaning of the pups at three weeks of age.

3) Weaned pups at 3 weeks of age: Tissue sampling for genotyping and/or identification via tail and earcut, respectively, under anesthesia (isoflurane).

Animals are killed by O2/CO2 method.

Welfare assessment:
Daily checks of the welfare of the mice on several common parameters (overall appearance, size, confirmation and growth, coat condition, behavior, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013

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

For these type of experiments statistical analysis is not performed since the purpose of the experiment is not to compare groups but to create viable novel mice lines for follow-up experiments. All techniques are state of the art and have been shown to be effective in generating GM mice with a smallest number of mice possible.

B. The animals

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

Mouse; Mus musculus: genetically modified and wild type adult mice. All mice are derived from the NIN, an establishment licensed by the NVWA, or from a registered commercial company.

For generation of GM mice we expect, based on our extensive experience, to generate max. 15 new lines over the next 5 years. For the creation of a new GM mouse lines we will use on average max. 150 mice (according to the besluit biotechnologie). Based on these numbers in total a maximum of 2250 mice will be required.

Welfare assesment: we expect to generate over the next 5 years 15 new GM lines for which we have to perform the welfare assesment. For 2 generations, 7 males and 7 females control and GM mice. We therfore need in total: 15 (new (compound) lines) * 2 (generation) * 28 ((7 male +7 female = 14 GM mice +

(7 male + 7 female = 14 control mice)) = 840 mice for the welfare assessment.

Taken together within the context of this procedure we need 2250 + 840 mice = 3090 mice

A large portion of the newly generated GG mice will be floxed mice, which have no phenotype by definition, and which are not part of the welfare assesment protocol. We will not breed new GM mice showing a hampered phenotype.

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

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

- (Vasectomized) males will also be used by the other groups of the NIN if required for their experiments, thereby reducing the number of (vasectomized) males used for the generation of GM mice.
  Mice used for welfare assessment, might be used for experiments described in procedures 3.4.4.1, 3.4.4.2 and 3.4.4.3.

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

☒ 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.

We start the generation of a new GM mouse line only after we are convinced that based on molecular screens in human tissue or animals (procedure 3.4.4.1), and in vitro experiments (procedure 3.4.4.2) the creation of a new line is essential for in vivo functional and mechanistic studies. Animal studies are essential unavoidable if we want to obtain comprehensive knowledge on the function of specific genes in processes of neuroregeneration and plasticity. The CRISPR/Cas9 system allows us, if required, to genetically modify multiple (that is up to 5 different) genes in a single experiments. This may strongly reduce the number of mice used for the generation and/or breeding of these GM mice.

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

All possibilities to reduce pain, fear or suffering will be used. There are no negative environmental effects; all mice will be housed under well-controlled DM1 conditions.

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.

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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.

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

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

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

Animals involved in welfare assessment (n = 840) will experience no pain. Animals involved in the generation of TG lines (n=2250) will experience pain as a consequence of the procedures and they receive adequate anaesthesia and analgesia.

I. Other aspects compromising the welfare of the animals

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

We do not expect to find additional adverse effect. This is the direct result of how we create our constructs for the generation of GM mice.

Explain why these effects may emerge.

We do not expect to find other adverse effect.

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

Daily monitoring of our mice; immediate action will be taken immediately if unexpectedly any adverse effect will become visible.

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.

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

Indicate the likely incidence.

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').

Vasectomized males: moderate
Donors: moderate 100%
Foster mothers: moderate 100%
GM mice: no to mild 100% (welfare assessment).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

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

The donor females will be killed as part of the experiments.
The foster females will be killed after the experiment (at the stage of weaning of the pups).

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.

☒ Yes

Type dierproef 1

Groep 1a	> ratten – adult – matig ongerief	240
Groep 1b	> muizen – adult – matig ongerief	240

Type dierproef 2

Groep 2a	> ratten – adult – licht ongerief	125 + 100
Groep 2b	> ratten – embryo's – licht ongerief	1000
Groep 2b	> ratten – pups (P1-P7) – licht ongerief	375
Groep 2d	> muizen – adult – licht ongerief	125 + 100
Groep 2e	> muizen – embryo's – licht ongerief	1000
Groep 2f	> muizen – P1-P7 – licht ongerief	375

Type dierproef 3

Groep 3a	> ratten – adult – matig ongerief	900
Groep 3b	> muizen – adult – matig ongerief	900

Type dierproef 4

Groep 4a	> ratten – adult – licht ongerief	460
Groep 4b	> muizen – adult – licht ongerief	180

Type dierproef 5

Groep 5a	> muizen – adult – licht ongerief	840	(welfare assessment)
Groep 5b	> muizen – adult –matig ongerief	2250	(generatie transgenen)

Cumulatief ratten: 3200
Ratten licht: 125 + 100 + 1000 + 375 +460 = 2060 => 65%
Ratten matig: 240 + 900 = 1140 => 35%

Cumulatief muizen: 6010
Muizen licht: 125 + 100 + 1000 + 375 +180 + 840 = 2620 => 44%
Muizen matig: 240 + 900 + 2250 = 3390 => 56%

Centrale Commissie Dierproeven

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Centrale Commissie Dierproeven

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www.centralecommissiedierproeven.nl

T 0900-2800028 (10 ct /min)

ZBO-CCD@minez.nl

Onze referentie
Aanvraagnummer
AVD801002015104

Uw referentie
-

Bijlagen
1

Datum 24 juni 2015
Betreft Beslissing Aanvraag projectvergunning dierproeven

Geachte [redacted]

Op 22 mei 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven digitaal ontvangen. Het gaat om uw project "Developing strategies to promote repair or plasticity of the central and peripheral nervous system" met aanvraagnummer AVD801002015104. Wij hebben uw aanvraag beoordeeld.

Op 12 juni 2015 heeft u uw aanvraag aangevuld op basis van door het secretariaat van de CCD gestelde vragen.

Beslissing
Wij keuren uw aanvraag onder voorwaarde goed op grond van artikel 10a van de Wet op de dierproeven (hierna de wet). U kunt met uw project "Developing strategies to promote repair or plasticity of the central and peripheral nervous system" starten. De vergunning wordt afgegeven van 1 juli 2015 tot en met 30 juni 2020.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC-KNAW gevoegd. 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. Wij nemen dit advies van de commissie over, inclusief de daaraan ten grondslag liggende motivering. Dit advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving liggen ten grondslag aan dit besluit.

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 gegevens in het colofon.

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).

Met vriendelijke groet,

De Centrale Commissie Dierproeven
namens deze:

[redacted]

ir. G. de Peuter
Algemeen Secretaris

Dit besluit is genomen met inachtneming van het Besluit mandaat, volmacht en machtiging van de Centrale Commissie Dierproeven CCD 2014 zoals de Centrale Commissie Dierproeven heeft vastgesteld op 19 december 2014, ref 2014-04 en is gepubliceerd in de Staatscourant van 2 januari 2015, Nr. 163

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: KNAW, Nederlands Herseninstituut
Adres: Postbus 19121
Postcode en woonplaats: 1000 GC Amsterdam
Deelnemersnummer: 80100

deze projectvergunning voor het tijdvak 1 juli 2015 tot en met 30 juni 2020, voor het project "Developing strategies to promote repair or plasticity of the central and peripheral nervous system" met aanvraagnummer AVD801002015104, volgens advies van dierexperimentencommissie DEC-KNAW.
De functie van de verantwoordelijk onderzoeker is group leader, group regeneration of sensory motor systems.
De aanvraag omvat de volgende bescheiden:

1. een aanvraagformulier projectvergunning dierproeven, ontvangen op 22 mei 2015.
2. de bij het aanvraagformulier behorende bijlagen:
   a. Projectvoorstel, zoals ontvangen bij digitale indiening op 22 mei 2015;
   b. Niet-technische Samenvatting van het project, zoals ontvangen bij digitale indiening op 22 mei 2015;
   c. Advies van dierexperimentencommissie d.d. 22 mei 2015, ontvangen op 22 mei 2015.
   d. Aanvullingen ontvangen op 12 juni 2015

Dierproeven

Naam dierproef	**Diersoort**	**Aantal dieren**	**Ernst**
Dierproef 1: Molecular screens: Sacrifice of mice or rats following a lesion and/or intervention with a viral vector to obtain neural tissue for molecular screens	Ratten- adult Muizen -adult	240 240	Matig Matig
Dierproef 2: Sacrifice of embryos of rats or mice or of adult rats or mice to obtain tissues for cell culture and bioassays.	Ratten –adult Ratten-embryo Ratten- P1-P7 Muizen –adult Muizen-embryo Muizen- P1-P7	225 1000 375 225 1000 375	Licht Licht Licht Licht Licht Licht
Dierproef 3: Injury models (functional and histological analysis): Injection of cells or viral vectors in lesioned animals.	Ratten- adult Muizen -Adult	900 900	Matig Matig
Dierproef 4: Testing of the quality of viral vector batches	Ratten –Adult Muizen -Adult	460 180	Licht Licht
Dierproef 5: Monitoring and generation of novel genetically modified mice	Muizen- adult *Welfare assessment* Muizen –adult *Genereren transgenen*	840 2250	Licht Matig

Aanvullende voorwaarden:
In Artikel 10, eerste lid, onder a, Wet op de dierproeven, 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 overleg met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarden wijzigen of intrekken.

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 van Economische Zaken 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 13c 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 13d 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.

[redacted]

Van: ZBO-CCD
Verzonden: vrijdag 22 mei 2015 14:32
Aan: 'secretariaat DEC'
CC: [redacted]
Onderwerp: ontvangstbevestiging AVD801002015104
Bijlagen: ontvangsbevestiging aanvraag projectvergunning dierproeven AVD 801002015104.pdf

Geachte heer/mevrouw,

Hierbij zenden wij u per mail een ontvangstbevestiging AVD/801002015104: Ontwikkeling van strategieën om zenuwweefselregeneratie te bevorderen.
Deze zal ook per post worden verzonden.

Met vriendelijke groet,

Centrale Commissie Dierproeven www.zbo-ccd.nl
Nationaal Comité advies proefdierbeleid

Postbus 20401 | 2500 EK | Den Haag

[redacted]

Van: ZBO-CCD
Verzonden: vrijdag 5 juni 2015 15:40
Aan: [redacted]
Onderwerp: tekstuele aanpassing AVD801002015104

Geachte meneer [redacted]

Zojuist heb ik gevraagd om enkele tekstuele verduidelijking voor projectaanvraag: Developing strategies to promote repair or plasticity of the central and peripheral nervous system" met nummer AVD 801002015104 . Omdat dit om enkel tekstuele verduidelijking gaat en niet om inhoudelijke vraagstelling wordt deze mail parallel aan aanvrager en DEC gestuurd,

Vraag:
In de beschrijving dierproeven 3.4.4.3 ; Injury models: Bij punt B beschrijft u in de eerste alinea een aantal van 750 genetisch gemodificeerde of WT muizen. Verderop in de tekst berekend u voor de injury models 900 ratten en 900 muizen. Het wordt uit de tekst niet helemaal helder of het totale aantal 750 GGO muizen + 900 ratten + 900 muizen is. Kunt u dit toelichten en als nodig in de tekst aanpassen?

In beschrijving dierproeven 3.4.4.4; testing of the quality of viral vector batches, zit een tekstuele inconsequentie: bij punt A beschrijft u dat de handelingen "without surgically inflicted damage" plaatsvinden . Bij punt D refereert u aan "all surgical procedures" . Kunt u bevestigen dat deze handelingen zonder chirurgie plaatsvinden? Mocht er wel chirurgie noodzakelijk zijn dan zou dit consequenties voor de ongerief inschatting "licht" kunnen hebben, kunt u dit toelichten?

Vriendelijke groet, [redacted]

Centrale Commissie Dierproeven
www.zbo-ccd.nl
.....
Postbus 20401 | 2500 EK | Den Haag
.....

[redacted]

Van: ZBO-CCD
Verzonden: donderdag 11 juni 2015 14:47
Aan: [redacted]
Onderwerp: FW: AVD801002015104 tekstuele uitleg/aanpassing

Geachte [redacted]

In aanvulling op onderstaande mail betreffende uw project: Developing strategies to promote repair or plasticity of the central and peripheral nervous system" met nummer AVD 801002015104. Zijn er nog een paar vragen. Excuses dat deze beoordeling gefaseerd gaat, hopelijk is het voor u nog mogelijk de antwoorden in een keer te doen en hebben deze berichten elkaar niet gekruist.

U beschrijft het gebruik van ratten en muizen, de CCD hecht eraan het aantal in voorraad gedode dieren terug te dringen en heeft daarom de vraag of u in zijn algemeenheid kunt toelichten of u (behalve voor de fok) ; voor de experimentele procedures beide geslachten kunt gebruiken of in het geval u 1 geslacht gebruikt motiveren waarom dit is?

In Bijlage 3.4.4.5. beschrijving dierproeven is punt H. niet volledig ingevuld, wij interpreteren dit als dat er NO in plaats van YES aangekruist had moeten worden.

Met vriendelijke groet [redacted]

Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl
.....
Postbus 20401 | 2500 EK | Den Haag
.....

Van: ZBO-CCD
Verzonden: vrijdag 5 juni 2015 15:33
Aan: [redacted]
Onderwerp: AVD801002015104 tekstuele uitleg/aanpassing

Geachte [redacted]

Uw aanvraag getiteld ; Developing strategies to promote repair or plasticity of the central and peripheral nervous system" met nummer AVD 801002015104 is ontvangen en in behandeling genomen. Er zijn twee tekstuele onduidelijkheden in de bijlage dierproeven waarvan we u willen vragen deze te verduidelijken en aan te passen zodat het aantal dieren en ongerief inschatting met elkaar in overeenstemming is.

In de beschrijving dierproeven 3.4.4.3 ; Injury models: Bij punt B beschrijft u in de eerste alinea een aantal van 750 genetisch gemodificeerde of WT muizen. Verderop in de tekst berekend u voor de injury models 900 ratten en 900 muizen. Het wordt uit de tekst niet helemaal helder of het totale aantal 750 GGO muizen + 900 ratten + 900 muizen is. Kunt u dit toelichten en als nodig in de tekst aanpassen?

In beschrijving dierproeven 3.4.4.4; testing of the quality of viral vector batches, zit een tekstuele inconsequentie: bij punt A beschrijft u dat de handelingen "without surgically inflicted damage" plaatsvinden . Bij punt D refereert u aan "all surgical procedures" . Kunt u bevestigen dat deze handelingen zonder chirurgie plaatsvinden? Mocht er wel chirurgie noodzakelijk zijn dan zou dit consequenties voor de ongerief inschatting "licht" kunnen hebben, kunt u dit toelichten?

Met vriendelijke groet, [redacted]

Centrale Commissie Dierproeven
www.zbo-ccd.nl
.....
Postbus 20401 | 2500 EK | Den Haag
.....

[redacted]

Van: secretariaat DEC [redacted]
Verzonden: vrijdag 12 juni 2015 13:28
Aan: [redacted]
CC: [redacted]
Onderwerp: AVD-801002015-104 tekstuele uitleg/aanpassing

Categorieën: [redacted]

Geachte mevrouw [redacted]

Namens [redacted] heb ik zojuist via Webftp een aantal files gestuurd met een brief met de antwoorden op de gestelde vragen alsmede een bijstelling van een aantal bijlagen en een tabel met een overzicht van het aantal dieren.

Met vriendelijke groeten,

[redacted] DEC-KNAW

From: [redacted]
Sent: Thursday, June 11, 2015 2:49 PM
To: secretariaat DEC
Subject: FW: AVD801002015104 tekstuele uitleg/aanpassing

Geachte heer [redacted]

Onderstaande mail is zojuist aan de onderzoeker verzonden met het verzoek op het punt van het gebruik van beide geslachten nog een toelichting te geven.

Vriendelijke groet, [redacted]

Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl
.....
Postbus 20401 | 2500 EK | Den Haag
.....

Van: ZBO-CCD
Verzonden: donderdag 11 juni 2015 14:47
Aan: [redacted]
Onderwerp: FW: AVD801002015104 tekstuele uitleg/aanpassing

Geachte [redacted],

In aanvulling op onderstaande mail betreffende uw project: Developing strategies to promote repair or plasticity of the central and peripheral nervous system" met nummer AVD 801002015104. Zijn er nog een paar vragen. Excuses dat deze beoordeling gefaseerd gaat, hopelijk is het voor u nog mogelijk de antwoorden in een keer te doen en hebben deze berichten elkaar niet gekruist.

U beschrijft het gebruik van ratten en muizen, de CCD hecht eraan het aantal in voorraad gedode dieren terug te dringen en heeft daarom de vraag of u in zijn algemeenheid kunt toelichten of u (behalve voor de fok) ; voor de

experimentele procedures beide geslachten kunt gebruiken of in het geval u 1 geslacht gebruikt motiveren waarom dit is?

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Met vriendelijke groet [redacted]

Centrale Commissie Dierproeven
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.....

Van: ZBO-CCD
Verzonden: vrijdag 5 juni 2015 15:33
Aan: [redacted]
Onderwerp: AVD801002015104 tekstuele uitleg/aanpassing

Geachte [redacted]

Uw aanvraag getiteld ; Developing strategies to promote repair or plasticity of the central and peripheral nervous system" met nummer AVD 801002015104 is ontvangen en in behandeling genomen. Er zijn twee tekstuele onduidelijkheden in de bijlage dierproeven waarvan we u willen vragen deze te verduidelijken en aan te passen zodat het aantal dieren en ongerief inschatting met elkaar in overeenstemming is.

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In beschrijving dierproeven 3.4.4.4; testing of the quality of viral vector batches, zit een tekstuele inconsequentie: bij punt A beschrijft u dat de handelingen "without surgically inflicted damage" plaatsvinden . Bij punt D refereert u aan "all surgical procedures" . Kunt u bevestigen dat deze handelingen zonder chirurgie plaatsvinden? Mocht er wel chirurgie noodzakelijk zijn dan zou dit consequenties voor de ongerief inschatting "licht" kunnen hebben, kunt u dit toelichten?

Met vriendelijke groet, [redacted]

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.....
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.....

[redacted]

Van: ZBO-CCD
Verzonden: donderdag 18 juni 2015 16:37
Aan: [redacted]
Onderwerp: betaling AVD2015104 mogelijk dubbel ontvangen

Geachte [redacted]

Voor uw project aanvraag hebben wij de leges ontvangen, maar uit onze administratie blijkt dat wij deze betaling mogelijk 2x hebben ontvangen op zowel 9-6-15 als op 16-6-15. Omdat ik geen gegevens van uw financiële afdeling heb hoop ik dat u deze vraag door kunt sturen.
Ik voeg de betalingskenmerken bij,

[redacted]

[redacted]

Vriendelijke groet, [redacted]

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