| | **Inventaris Wob-verzoek W17-09** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | **wordt verstrekt** | | | | **weigeringsgronden** | | | |
| **nr.** | **document** | **reeds openbaar** | **niet** | **geheel** | **deels** | **10.1.c** | **10.2.e** | **10.2.g** | **11.1** |
| | **NTS20171289** | | | | | | | | |
| 1 | Aanvraagformulier | | | | x | | x | x | |
| 2 | Projectvoorstel oud | | | | x | | | x | |
| 3 | Niet-technische samenvatting oud | | | | x | | | x | |
| 4 | Bijlage beschrijving dierproeven oud | | | | x | | | x | |
| 5 | DEC-advies | | | | x | | x | x | |
| 6 | Ontvangstbevestiging | | | | x | | x | x | |
| 7 | Verzoek aanvulling aanvraag | | | | x | | x | x | |
| 8 | Projectvoorstel nieuw | | | | x | | | x | |
| 9 | Bijlage beschrijving dierproeven nieuw | | | | x | | | x | |
| 10 | Niet-technische samenvatting nieuw | x | | | | | | | |
| 11 | Advies CCD | | x | | | | | | x |
| 12 | Beschikking en vergunning | | | | x | | x | x | |

Centrale Commissie Dierproeven

# Aanvraag
## Projectvergunning Dierproeven
*Administratieve gegevens*

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

## 1 Gegevens aanvrager

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

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

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

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

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

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

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

| | | |
|---|---|---|
| (Titel) Naam en voorletters | | ☐ Dhr. ☐ Mw. |
| Functie | | |
| Afdeling | | |
| Telefoonnummer | | |
| E-mailadres | | |

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

## 2 Over uw aanvraag

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

## 3 Over uw project

| | | | |
|---|---|---|---|
| 3.1 | Wat is de geplande start- en einddatum van het project? | Startdatum | 1 september 2017 |
| | | Einddatum | 31 augustus 2022 |
| 3.2 | Wat is de titel van het project? | Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model | |
| 3.3 | Wat is de titel van de niet-technische samenvatting? | Het voorkomen van [redacted] stoornissen in de ziekte van Parkinson: een nieuw preklinisch model | |
| 3.4 | Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt? | Naam DEC | DEC Vrije Universiteit / VU Medisch Centrum |
| | | Postadres | [redacted]<br>Amsterdam \| Nederland |
| | | E-mailadres | [redacted] |

## 4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1035

☐ 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*

* Wanneer de factuur direct naar de financiële afdeling van de VU of het VUmc dient te gaan moet hier een inkooporder nummer en factuuradres worden toegevoegd door de onderzoekers, graag van te voren afstemmen met de financiële afdeling.

Inkooporder nummer:
Factuuradres:

Graag verzoeken we de CCD om het bovenstaande inkooporder nummer aan de factuur toe te voegen en de factuur te versturen naar het factuuradres.

## 5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☒ Melding Machtiging

☐

## 6 Ondertekening

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

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

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

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

| | |
|---|---|
| Naam | [redacted] |
| Functie | [redacted] |
| Plaats | Amsterdam |
| Datum | 03 - 04 - 2017 |
| Handtekening | [redacted] |

2.

Centrale Commissie Dierproeven

# Form
## Project proposal

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

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 11400 |
| 1.2 | Provide the name of the licenced establishment. | VUmc |
| 1.3 | Provide the title of the project. | Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model |

## 2 Categories

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

- ☒ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

## 3 General description of the project

### 3.1 Background

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

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

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease. It is a progressive disorder, characterized by severe motor symptoms and motor dysfunctions and affects approximately 2% of the population. Whereas our understanding of the etiology of Parkinson's disease

has increased over the last decade, to date there is no curative therapy. One of the underlying causes of Parkinson's disease is degeneration of the brain's dopamine neurotransmitter system. Therefore in Parkinson's disease many of the motor symptoms can be improved by dopamine replacement therapy to boost the activity of the dopamine system. This dopamine replacement therapy consists of chronic treatment with the dopamine precursor levodopa and chronic treatment with dopamine receptor agonists such as pramipexole, or a combination of levodopa and dopamine receptor agonist.

Unfortunately, frequent side-effects of these dopamine replacement therapies are [redacted] disorders following onset of the treatment and they pose a major burden on patients, their families and caregivers. It is estimated that [redacted] disorders develop in 15% to even up to 25% of patients and commonly include pathological gambling, compulsive sexual behaviour, compulsive buying and binge eating [1,2]. Results of cross-sectional studies have revealed some generic factors associated with an increased risk for [redacted] disorders, e.g. young age of onset, trait impulsivity, (family)history of addiction and depression. Nonetheless, current knowledge of the characteristics related to increased susceptibility of developing [redacted] disorders is insufficient to adequately predict or prevent these disorders in daily clinical practice. More importantly, the mechanisms underlying the development and the course of [redacted] disorders in Parkinson's disease are poorly understood and cannot be directly manipulated in clinical populations.

Also, there is no effective therapy to prevent the onset of [redacted] disorders arising with dopamine therapies. Therefore having access to a translational animal model, which unravels the mechanisms and reliably predicts the risk for [redacted] disorders when treated with dopamine replacement therapy, will be a valuable model to develop novel or to refine existing pharmacotherapies for Parkinson's disease that do not induce [redacted] disorders. Moreover, the availability of such a model will also allow testing of adjuvant pharmacotherapies that alleviate [redacted] disorders.

This project aims to **develop a novel translational animal model for Parkinson's disease mimicking the risk to develop [redacted] disorders upon dopamine therapy and unraveling the underlying brain mechanisms**. As such, this novel animal model may be utilized as a preclinical tool to facilitate and aid the development of novel pharmacotherapies for Parkinson's disease that do not induce [redacted] disorders. Establishing such a model will have a great impact on the field given the sense of urgency from both clinicians as well as patients. In addition, if this novel model is successful another aim is to test whether adjuvant therapy reduces the risk to develop [redacted] disorders.

Previous and current work using different approaches to mimic Parkison's disease pathology in animals, including 6-OHDA lesions and alpha-synuclein viral overexpression, have indeed demonstrated development of [redacted] deficits and altered responsivity to dopamine therapies. However, the interventions in such animal models are irreversible, invasive by inducing permanent brain damage and lack specific or temporal control over relevant brain pathways.

To address these limitations, this project will take advantage of recent technological advances to highly selectively and temporally-precise manipulate and uncover crucial brain pathways that could drive the risk for [redacted] disorder development in Parkinson's disease. This will be achieved by the novel innovative chemogenetic approach called designer-receptors exclusively activated by designer drugs (DREADD) to mimic Parkinson-related pathology in cortico-striato-thalamo-cortical brain pathways, since these pathways are thought to underlie the risk to develop [redacted] disorders [3]. The DREADD approach will be further specified in **paragraph 3.4** (Research Strategy). First, we will combine DREADD with dopamine therapy in the same animals performing cognitive tasks that capture [redacted] deficits as a proxy to mimic clinical [redacted] disorders in Parkinson's disease. Second, upon establishing this animal model, we will study the effects of adjuvant pharmacotherapy to ameliorate dopamine therapy-induced [redacted] disorders.

[1] Vriend C, et al (2014) Neurosci Biobehav Rev 38: 60-71.
[2] Weintraub D, et al (2015) Mov Disord 30:121-127.
[3] Gerfen CR and Surmeier DJ (2011) Ann Rev Neurosci 34: 441-466.

### 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 purpose and objective** of this project is to develop a novel translational animal model that mimics the risk of developing [redacted] disorders after dopamine therapy as a preclinical animal model to facilitate and aid the development of novel pharmacotherapies without the risk of disturbed [redacted]

In line with this main objective, the specific sub-objectives are:

**Objective 1a: To establish differential effects of dopamine agonist therapy on DREADD modulation of cortico-striato-thalamo-cortical brain pathways as a proxy and proof-of-concept to mimic clinical [redacted] disorders in Parkinson's disease**

For this sub-objective, we will address the causal involvement of manipulating specific neural brain pathways in the onset of [redacted] disturbances. Furthermore, we will address the question which dopamine agonist monotherapy, levodopa or pramipexole, is most likely to induce deficits in [redacted] after subchronic treatment. Levodopa and pramipexole are different classes of dopamine therapy and clinically might possess subtle differences in the risk for onset of [redacted] disorder. Therefore, it is important to test both compounds in our novel animal model, since our model allows to further dissect the causal effects of levodopa or pramipexole treatment and their differences on deficits in [redacted]

**Objective 1b: Effects of adjuvant pharmacotherapy to suppress dopamine therapy-induced [redacted] deficits**

For this sub-objective, we will address the question whether adjuvant treatment, on top of the dopamine therapy that has the most pronounced risk to develop impulsivity, is able to suppress these effects of dopamine therapy on [redacted] deficits. More specifically, we will focus here on three different classes of clinically-used drugs as adjuvant therapies, namely 1) the antidepressant drug and selective serotonin reuptake inhibitor citalopram; 2) the anti-ADHD drug and selective norepinephrine reuptake inhibitor atomoxetine; and 3) the opioid antagonist naltrexone. These drugs are selected, since they have demonstrated to possess beneficial effects on [redacted] under different circumstances in both rats and humans, and therefore seem promising candidates as an adjuvant therapy in the clinic. However, none of these three classes of drugs have the indication Parkinson's disease, and moreover to our knowledge have not been prescribed as adjuvant therapy to dopamine replacement therapy. Therefore, our study will provide important information regarding the effectivity and applicability of this approach.

The work described in this project will be funded by an international peer-reviewed research grant from a non-profit organization that funds research into the etiology and treatment of neurodegenerative disorders including Parkinson's Disease.

The main objective and sub-objectives of the project are **achievable**, since:
1) we have ample experience using DREADD as a methodology for neural interventions that are crucial to increase our understanding of the brain pathways involved
2) we have a strong track-record and experience in translational cognitive tasks capturing [redacted] deficits, documented by many scientific papers in international journals
3) our facility has adequate numbers of operant chambers to run the cognitive tasks and housing facilities for the project
4) in addition to research technicians who will run part of the experiments, the research grant allows hiring full time staff at the postdoctoral level to complete all of these experiments within the time-frame of the project
5) all of the equipment needed for DREADD approaches is already present and the viral vectors needed are commercially available, in addition to the pharmacotherapies which are commercially available

### 3.3 Relevance

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

**Scientific relevance**
Randomized clinical trials are highly costly and lengthy in duration, therefore having access to a translational preclinical model which reliably predicts [redacted] disturbances following dopamine therapy will be a valuable model that will aid the development of novel or refinement of existing

pharmacotherapies for Parkinson's disease without the risk to induce deficits in [redacted]
Furthermore, the DREADD approach of circuit-relevant and temporal specific manipulation of neural pathways will allow unique insights into the aetiology and mechanisms of [redacted] disorder development beyond the currently existing Parkinson's disease animal models and beyond what can be studied in clinical populations.

**Social relevance**
Despite the clinical efficacy of dopamine therapy in ameliorating motor symptoms in Parkinson's disease, there is no effective intervention to prevent the onset of potential deficits in [redacted] arising with pharmacotherapy. Given the tremendous burden of [redacted] disorders on patients, their families and caregivers, novel (adjuvant) pharmacotherapies that do not cause disturbances in [redacted] are highly needed. The experiments described in this project are intended to aid the development of such novel (adjuvant) pharmacotherapies for Parkinson's disease.

## 3.4 Research strategy

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

**Objective 1a: To establish differential effects of dopamine agonist therapy on DREADD modulation of cortico-striato-thalamo-cortical brain pathways as a proxy and proof-of-concept to mimic clinical [redacted] disorders in Parkinson's disease**

To address **objective 1a** and to mimic altered cortico-striato-thalamo-cortical brain pathway functioning as a way to mimic Parkinson pathology, we will employ an innovative retro-DREADD procedure [1], which permits remote control of specific neural pathways. Essentially, DREADDs (engineered G protein-coupled receptors) are targeted to specific neuronal populations using viral-vector mediated gene transfer and can be transiently activated by a synthetic pharmacological agent, that is otherwise inert. As such, DREADD provides the unique opportunity to selectively and transiently activate or silence these brain pathways and to determine their functional relevance in freely moving animals performing in cognitive tasks. This will allow unique insights into the aetiology and mechanisms of [redacted] disorder development beyond the currently existing Parkinson's disease animal models and beyond what can be studied in clinical populations.

Given the specific importance of the ventral cortico-striato-thalamo-cortical circuit in onset of [redacted] disorders, we use a dual-virus strategy to target defined neural circuits. For this, we first inject into the target region (e.g. NAc) a viral vector that is transported in a retrograde direction back to the projecting region (e.g. mPFC). In the projection region, we inject another virus that encodes the DREADD, but does not express this receptor unless the other viral vector is present. Therefore, DREADD expression is restricted to neurons defined by their circuit connectivity. With this approach, we can study the contribution of specific cortico-striato-thalamo-cortical brain pathways in mediating the effect of dopamine therapy on the onset of [redacted] deficits. The projections we aim to study are depicted in **Figure 1**, and in different groups of rats we will inject a combination of the excitatory and inhibitory DREADD virus [2] into the projection brain area and inject the other viral vector in the target region of this brain area within the same animals.

Following inhibitory or excitatory DREADD expression, animals will be trained in translational cognitive tasks including the rodent stop-signal task measuring cognitive control and a rodent gambling task measuring risk-based decision-making as a proxy of [redacted] deficits. Upon stable baseline performance, we will probe altered brain pathway functioning on cognitive control/ risk-based decisions and motor function by DREADD activation using systemic administration of DREADD ligands to either inhibit or activate the pathways under investigation. These experiments will unravel whether manipulation of specific brain pathways to mimic Parkinson pathology already induces [redacted] deficits.

Following these tests, while still trained in the cognitive tasks on a daily basis, dopamine therapy will start and animals will receive subchronic levodopa treatment or pramipexole treatment for 21 days and on days 1, 7, 14 and 21 we will additionally administer the DREADD ligands to assess the interaction

between dopamine therapy and altered cortico-striato-thalamo-cortical brain pathway connectivity on [redacted] deficits.

Based on the outcomes of these experiments we will select the specific cortico-striato-thalamo-cortical brain pathways which in combination with dopamine therapy (either levodopa or pramipexole treatment) are most likely to induce deficits in [redacted] in order to further address **objective 1b**.

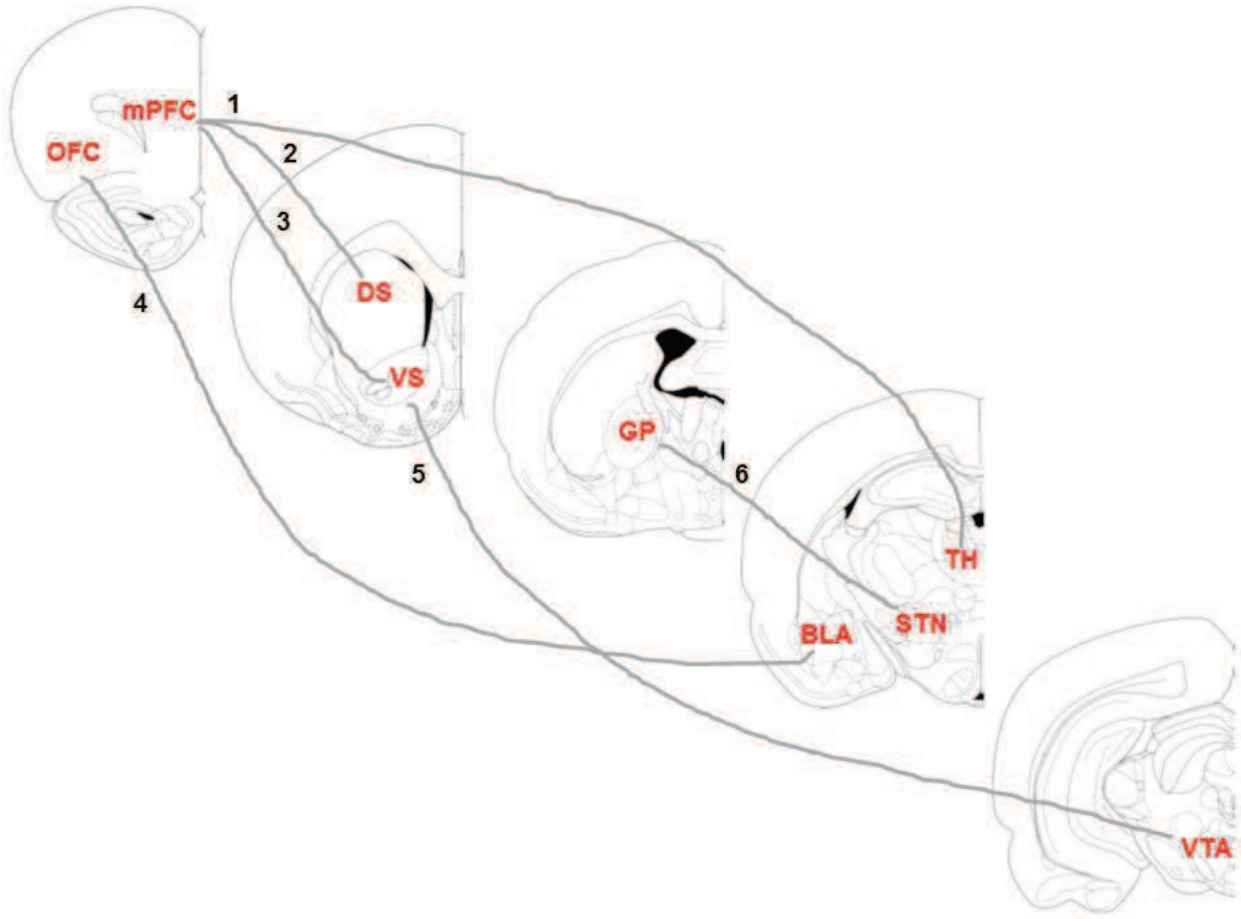


**Figure 1.** Schematic depiction of the different cortico-striato-thalamo-cortical brain pathways that will be targeted in the project. 1) thalamus to mPFC; 2) mPFC to dorsal striatum, DS; 3) mPFC to ventral striatum, VS; 4) orbitofrontal cortex, OFC, to basolateral amygdala, BLA; 5) ventral tegmental area, VTA, to VS; 6) subthalamic nucleus, STN, to globus pallidus, GP. Depicted are coronal sections of the rat brain [adapted from 3].

**Objective 1b: Effects of adjuvant pharmacotherapy to suppress dopamine therapy-induced [redacted] deficits**

In this set of experiments ,we will assess the effects of adjuvant pharmacotherapy on levodopa- or pramipexole-induced disturbances in [redacted] in the pathways that have been found to be most importantly involved in the onset of these disturbances. We will here focus on the SSRI citalopram, the SNRI atomoxetine and the opioid antagonist naltrexone as adjuvant therapies, given 1) the beneficial effects of these compounds on [redacted] and 2) the fact that these compounds are in clinical use. If successful, this will indicate that the model has immense potential for follow-up experiments to search for novel pharmacotherapies to treat Parkinson's disease without the risk of developing deficits in [redacted]

[1] Marchant NJ, et al (2016) Neuropsychopharmacology 41: 402-409.
[2] Vardy E, et al (2015) Neuron 86: 936-946.

[3] Paxinos and Watson (1998) Academic Press Ltd.

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.

As described in **paragraph 3.4** (Research Strategy), all animals in the experiments described in **both objective 1a and objective 1b** will undergo the same procedures and will be tested in each of the different components of the project following the same time-line, namely 1) viral expression of inhibitory or excitatory DREADD will be established in all animals; 2) animals will be trained in the cognitive tasks until baseline performance; 3) animals will receive pharmacological treatments with DREADD ligands and dopamine therapy (**objective 1a**) or DREADD ligands, dopamine therapy and adjuvant therapy (**objective 1b**) and 4) ex vivo analyses following behavioural testing.

Ad 1. Viral DREADDs expression:
To incorporate inhibitory or excitatory DREADDs to manipulate relevant brain pathways, viral vectors that travel along axons need to be expressed in relevant brain regions. For this, we will perform intracranial viral surgery prior to the cognitive testing. Then following recovery, animals will be trained in the cognitive tasks as described below. The primary outcome measures of viral DREADDs expression are sufficient levels of viral vector expression. This will be determined in study 1.

Ad 2. Cognitive tasks measuring [redacted] deficits:
Following recovery of the intracranial viral surgery for DREADD and viral vector expression in the selected brain pathways, animals will be trained daily during weekdays in either the rodent stop-signal task or the rodent gambling task until stable baseline performance is achieved. Training of these tasks takes place in operant chambers which are computer-controlled boxes containing levers, nosepoke units or other operanda that the subjects can operate, and visual stimuli and a pellet dispenser that can deliver small highly palatable food pellets. Both of the employed tasks are rodent analogues of existing human neuropsychological tasks, i.e. the stop-signal task and IOWA gambling task.
Briefly, in the rodent stop-signal task subjects are trained to make a response as quickly as possible once a go stimulus (=visual stimulus) is presented to earn a reward. Occasionally, this go stimulus is followed by a stop stimulus (=auditory stimulus) instructing the subject to withhold making a response. Successful inhibition upon stop stimulus presentation also leads to reward. Behavioural performance on these latter stop stimulus trials reflects response inhibition capacities and is the primary measure of the task. Poor response inhibition capacities in the task reflect [redacted] deficits.
In the rodent gambling task, that measures risky decision-making, rats are allowed to choose between 4 different response options, each one of them is associated with a different size of palatable food pellets and aversive wait periods to receive these pellets. There are 2 safe response options which are associated with low numbers of food pellets (1 or 2 pellets) and short wait periods. In contrast, the risky response options are associated with higher number of food pellets (3 or 4 pellets) and long wait periods. Optimal performance in this task would be to prefer the safe response options, which result in a nett larger win of food pellets. A preference for the risky response options results in a nett lower win of food pellets and reflects [redacted] deficits.
Primary outcome measure of these cognitive tasks is stable baseline performance in terms of response inhibition (stop-signal task) and risky decision-making (gambling task).

Ad 3. Pharmacological manipulation with DREADD ligands, dopamine therapy and adjuvant pharmacotherapy:
Upon stable baseline performance, before and during dopamine therapy we will probe altered brain pathway functioning on cognitive control/ risk-based decisions and motor function by DREADD activation using systemic administration of DREADD ligands. For subchronic dopamine therapy with either levodopa or pramipexole (**objective 1a**) or the combination of dopamine therapy with adjuvant therapy (**objective 1b**), animals will receive a single dose of the drug which will be administered peripherally once daily before testing in the cognitive tasks for 21 subsequent days. For both levodopa and pramipexole as DRT both a low and high dose will be tested in different groups using a between-subjects design. Adjuvant therapy consists of either the SSRI citalopram, the SNRI atomoxetine and the opioid

antagonist naltrexone.

In these experiments, the primary outcome measures are the effects of DREADD manipulation and dopamine therapy alone or combined with adjuvant pharmacotherapy on response inhibition (stop-signal task) and risky decision-making (gambling task).

Ad 4. Ex vivo analyses
We will sacrifice the animals at the end of the behavioural phase of experiments for subsequent ex vivo analyses. This in order to further unravel neurobiological changes that have occurred in the specific brain pathways. We will dissect brains to perform molecular biological or neurophysiological experiments aimed at understanding the neurobiological changes that have occurred in the selected brain pathways upon DREADD manipulation and their interaction with dopamine therapy. Molecular biological experiments will focus on changes in mRNA or protein levels of various neurotransmitter receptors such as, for instance, dopamine receptors as primary outcome measures. Neurophysiological experiments will focus on functional physiological changes in the selected brain pathways that were targeted with DREADD as primary outcome measures.

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.

The coherence between the different components and steps of the project is depicted in **Figure 2**.


***Figure 2.*** *Outline of decision points where animals will be used or not depending on the outcome of experiments. Note that only the maximum estimated numbers of animals within each study are depicted.*

**STUDY 1 (Milestone 1):**
In order to optimize the expression levels of the inhibitory or excitatory DREADD virus and viral vector in the target region, we will first conduct pilot studies, in which we intracranially express these viruses. These studies we will determine the optimal conditions for DREADD expression and viral vector expression in the different cortico-striato-thalamo-cortical pathways.

**STUDY 2 (Milestone 2):**
To address **objective 1a**, we will combine inhibitory and excitatory DREADDs in different brain pathways with either levodopa or pramipexole to develop a novel animal model to study deficits in [redacted] in Parkinson's disease. Thus this novel model aims to elucidate altered brain pathways that combined with dopamine therapy induce disturbed [redacted] as a proxy of [redacted] disorders in Parkinson's disease. Therefore, we will perform experiments described under this objective to pinpoint which combination of brain pathway DREADD manipulation and dopamine therapy is most powerful to evoke and to mimic [redacted] deficits.

**STUDY 3 (Milestone 3):**
To address **objective 1b**, we will test whether adjuvant pharmacotherapy (citalopram, atomoxetine and naltrexone) is capable of suppressing and preventing the onset of deficits in [redacted] after dopamine therapy. Here we will only study the DREADD brain pathways and dopamine therapy combinations that have induced [redacted] deficits. As such, the number of experiments and subjects required here depends on the results of the second series. We will test all three different adjuvant pharmacotherapies in these selected relevant DREADD brain pathways and dopamine therapy combinations.

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 | DREADD expression and pharmacotherapeutic interventions during behaviour |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |
| 10 | |

Centrale Commissie Dierproeven

# Format
## Niet-technische samenvatting

- Dit format gebruikt u om uw niet-technische samenvatting te schrijven
- Meer informatie over de niet-technische samenvatting vindt u op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

## 1 Algemene gegevens

| | | |
|---|---|---|
| 1.1 | Titel van het project | Het voorkomen van [redacted] stoornissen in de ziekte van Parkinson: een nieuw preklinisch model |
| 1.2 | Looptijd van het project | 5 jaar |
| 1.3 | Trefwoorden (maximaal 5) | Parkinson, [redacted] stoornissen, dopaminetekort behandeling, hersenen |

## 2 Categorie van het project

2.1 In welke categorie valt het project.

*U kunt meerdere mogelijkheden kiezen.*

- X Fundamenteel onderzoek
- X Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

## 3 Projectbeschrijving

3.1 Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)

De ziekte van Parkinson is een veelvoorkomende hersenaandoening die ongeveer 2% van de bevolking treft en die wordt gekenmerkt door ernstige motorische stoornissen veroorzaakt door tekorten in de boodschapperstof dopamine in de hersenen. Deze boodschapperstof dopamine is belangrijk in de communicatie tussen hersencellen. De symptomen van de ziekte van Parkinson worden na verloop van tijd steeds erger en behandeling van de symptomen bestaat meestal uit medicijnen die het tekort aan dopamine in de hersenen aanvullen. Als gevolg van deze behandeling krijgt een aanzienlijk deel van de patiënten te maken met ernstige psychiatrische afwijkingen zoals dwangmatig sexueel gedrag, dwangmatig koopgedrag en eetstoornissen. Deze ongewenste bijwerkingen zijn psychisch zeer belastend voor zowel de patiënt als voor de partner en de sociale omgeving. Deze afwijkingen worden

| | | |
|---|---|---|
| | | samengevat onder de noemer [redacted] stoornissen.<br><br>Neurobiologische kennis is noodzakelijk om de oorzaak van deze [redacted] stoornissen na dopaminetekort behandeling te begrijpen en te voorkomen.<br>Dit project richt zich op de hersencircuits die verantwoordelijk zijn voor het ontstaan van [redacted] stoornissen na dopaminetekort behandeling teneinde in de toekomst betere behandelingen te kunnen ontwikkelen. |
| 3.2 | Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang? | Uit dit project zal blijken welke hersengebieden verantwoordelijk kunnen zijn voor het ontstaan van [redacted] na behandeling van dopaminetekorten. Daarnaast zal worden onderzocht of het gelijktijdig gebruik van een ander medicijn een oplossing biedt voor [redacted] stoornissen. Deze kennis zal de ontwikkeling van nieuwe therapeutische behandelingen voor de ziekte van Parkinson zonder ontwikkeling van [redacted] stoornissen kunnen versnellen. |
| 3.3 | Welke diersoorten en geschatte aantallen zullen worden gebruikt? | Experimenten zullen worden uitgevoerd met ratten. Maximaal zijn 3140 dieren nodig. |
| 3.4 | Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren? | Tijdens een operatie onder adequate anesthesie krijgen de dieren een virale vector ingespoten in de hersenen zodat op een later moment hersencircuits zeer selectief gemanipuleerd kunnen worden en het effect op gedrag bestudeerd kan worden. De operaties, het bijkomen uit de narcose en het geven van de medicijnen kunnen tot tijdelijk ongerief leiden. |
| 3.5 | Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst? | Hersenoperatie: matig ongerief<br>Gedragstaken en geven medicijnen: licht ongerief<br><br>Alle dieren ondergaan matig ongerief |
| 3.6 | Wat is de bestemming van de dieren na afloop? | Aan het einde van de experimenten zullen de dieren worden gedood en zal hersenmateriaal gebruikt worden voor verder onderzoek. |

## **4** Drie V's

| | | |
|---|---|---|
| 4.1 | **Vervanging**<br>Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden. | Hersenaandoeningen zijn zeer complex en het zeer selectief manipuleren van hersenen teneinde oorzakelijke verbanden vast te stellen hoe [redacted] stoornissen ontstaan na dopamine behandeling is nog niet mogelijk bij mensen en maakt proefdieronderzoek noodzakelijk. Doordat gedragstesten worden uitgevoerd kan geen gebruik van celkweken worden gemaakt. |
| 4.2 | **Vermindering**<br>Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt. | De best gevalideerde gedragsmodellen voor [redacted] worden gebruikt en daarnaast wordt er een poweranalyse gebruikt om het aantal te gebruiken dieren tot een minimum te beperken.<br><br>Na elk experiment vindt er een afweging plaats (op basis van de verkregen resultaten) over het wel of niet uitvoeren van vervolgexperimenten. Zo zullen vervolgexperimenten alleen plaatsvinden als er een duidelijke relatie is gevonden tussen een bepaald hersengebied en [redacted] |

| | | |
|---|---|---|
| | | stoornissen. |
| 4.3 | **Verfijning**<br>Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project. | De rat is de meest gebruikte diersoort in modellen voor [redacted]. De gebruikte diermodellen voor [redacted] zijn direct afgeleid van neuropsychologische testen voor de mens en beschikken over een grote mate van voorspelbaarheid ten aanzien van het gedrag in mensen. |
| | Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden. | Er vinden dagelijkse welzijnsrapportages plaats waarbij het dier op uiterlijke gezondheidskenmerken en welzijn wordt gescoord. Daarnaast worden gedurende en na de hersenoperatie anesthesie en pijnstilling gebruikt om het ongerief van de ingrepen tot een minimum te beperken en worden duidelijk omschreven humane eindpunten toegepast. Operaties en biotechnische handelingen worden uitgevoerd door ervaren personeel aan de hand van gevalideerde protocollen en onder wettelijke toezicht. |

## **5** In te vullen door de CCD

| | |
|---|---|
| Publicatie datum | |
| Beoordeling achteraf | |
| Andere opmerkingen | |

# Appendix
## Description animal procedures

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

## 1 General information

| | | | |
|---|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 11400 | |
| 1.2 | Provide the name of the licenced establishment. | VUmc | |
| 1.3 | List the serial number and type of animal procedure.<br><br>*Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.* | Serial number | Type of animal procedure |
| | | 1 | DREADD expression and pharmacotherapeutic interventions during behaviour |

## 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.

The general outline of the experiments of the project is depicted below in **Figure 1**.

**Figure 1.** General outline of experiments. **STUDY 1:** Pilot studies for optimizing DREADD expression in selected brain pathways. **STUDY 2:** DREADD in selected brain pathways combined with dopamine therapy alone to study onset of deficits in [redacted] **STUDY 3:** DREADD in selected brain pathways combined with dopamine therapy + adjuvant therapy to ameliorate deficits in [redacted]

**STUDY 1. Pilot studies to optimize DREADD expression in selected brain pathways**

In order to develop a novel animal model mimicking the pathology of Parkinson's disease, we use a dual-virus strategy to target specific cortico-striato-thalamo-cortical brain pathways. For this, we first inject into the target region (e.g. NAc) a viral vector that is transported in a retrograde direction back to the projecting region (e.g. mPFC). In the projection region, we inject a virus that encodes the DREADD, but does not express this receptor the viral vector is present. Therefore, DREADD expression is restricted to neurons defined by their circuit connectivity. With this approach, we can study the contribution of different brain pathways in mediating the effect of DRT on ICD development.

Prior to conducting the behavioural experiments, we will optimize the viral expression patterns of the DREADD constructs in each of the selected brain pathways in pilot studies before we proceed with this pathway in the behavioural experiments. As shown in **Table 1**, for these pilot studies there are 2 Factors: brain pathway (6 pathways) X Virus (DREADD virus: inhibitory or excitatory).

During two surgeries, we will place the rat into the stereotaxic frame and use a needle attached to the stereotact to accurately inject the two viral vectors (DREADD and target region viral vector) into the specific brain regions (projection and target region). After recovery and a waiting period of approximately 4-8 weeks to allow sufficient DREADD expression, animals will be sacrificed to determine viral expression patterns in brain tissue.

**PRIMARY OUTCOME PARAMETERS:** The primary outcome of these pilot studies is the level of DREADD expression and target region viral vector expression in the selected brain pathways. These expression levels should be sufficient in order to continue with the behavioural experiments.

**Table 1.** Estimated animal numbers in STUDY 1, pilots for DREADD expression

| *STUDY 1* | *Virus injection* | *Test Drug* | *N per group* | *Total N per experiment* |
|---|---|---|---|---|
| Pilot infusions DREADD in selected brain pathways for sufficient expression levels | Inhibitory and excitatory DREADDs +target region viral vector | none | 10 | 2 DREADDs X<br>6 CSTC pathways X<br>10 rats =<br>**120 rats** |

**STUDY 2. Objective 1a: Differential effects of dopamine agonist therapy on DREADD modulation of cortico-striato-thalamo-cortical brain pathways as a proxy and proof-of-concept to mimic clinical [redacted] disorders in Parkinson's disease**

Here we will combine DREADD in different cortico-striato-thalamo-cortical circuits with either levodopa or pramipexole dopamine therapy in animals that are trained in the stop-signal task to measure response inhibition, or a gambling task to measure risky decision-making. This in order to unravel the underlying brain pathways and mechanisms as a novel model to mimic [redacted] disorders in Parkinson's disease.

Description of the cognitive tasks:
In Parkinson's disease, deficits in [redacted] are characterized by an inability to suppress certain (inappropriate) impulses that may lead to maladaptive behaviour including hypersexuality, compulsive

buying and gambling behaviour. To capitulate on these different clinical aspects using a translational approach we will employ 1) the stop-signal task to measure cognitive control and 2) the rodent gambling task to measure risky decision making. These tasks are instrumental learning tasks which take place in operant chambers outside of the home cage of the animals. Training in each of these tasks takes 2-3 months daily training per task and since both tasks measure different forms of [redacted] it is not feasible to test both tasks within the same individuals. Therefore separate groups of animals will be required for each task.

Ad 1) In laboratory settings response inhibition is an often used behavioural measure to assess cognitive control, that is the ability to control one's impulses. The stop-signal task measures response inhibition and requires subjects to inhibit inappropriate responses when certain cues, in this case stop-signals are provided. Briefly, in this task subjects are trained to make a response as quickly as possible once a go stimulus (=visual stimulus) is presented. Occasionally, this go stimulus is followed by a stop stimulus (=auditory stimulus) instructing the subject to withhold making a response. Behavioural performance on these latter stop stimulus trials reflects response inhibition capacities and is the primary measure of the task. Whereas the stop-signal task is designed to primarily tax response inhibition, the task also measures reaction latencies on go trials, prematurely expressed responses before go stimulus presentations and omitted trials. These parameters control for other behavioural alterations such as motivation to participate in the task (omitted trials), general deficits in inhibitory control (prematurely expressed responses) and changes in motor performance (reaction latencies and omitted trials).

Ad 2) The rodent gambling task will be employed to assess risky-decision making. This translational task is adopted from the Iowa gambling task and requires rats to choose between 4 different response options, each one of them is associated with a different size of palatable food pellets and aversive wait periods to receive these pellets. The safe response options are associated with low numbers of food pellets (1 or 2 pellets) and short wait periods, whereas the risky options are associated with higher number of food pellets (3 or 4 pellets) and long wait periods. Optimal performance in this task would be to prefer the safe response options, which result in a nett larger win of food pellets. A preference for the risky response options results in a nett lower win of food pellets and reflects [redacted] deficits.

Estimated number of animals

For the experimental groups receiving dopamine replacement therapy there are 5 Factors: brain pathway (6 pathways) X Virus (DREADD viral vectors: combination of inhibitory and excitatory) X dopamine replacement therapy (levodopa, pramipexole) X Dose (placebo, low dose, high dose) X cognitive task (stop-signal task, gambling task) as indicated in **Table 2**.

During surgery, we will place the rat into the stereotaxic frame and use a needle attached to the stereotact to accurately inject the two viruses (DREADD and target region viral vector) into the specific brain regions (projection and target region). Based on recent work showing successful combination of excitatory and inhibitory DREADDs by multiplexing these different DREADDs within individuals [1], we will also multiplex these DREADDs within the same individuals. This will lead to a tremendous **reduction** in the number of animals used in the project. After recovery, animals will be trained daily in the cognitive tasks and upon establishment of baseline performance, first tests with the DREADD ligands will be conducted in the cognitive tasks, followed by dopamine therapy in combination with DREADD ligands in the cognitive tasks.

*Control DREADD virus experiments:*

Only in the CSTC pathways in which we observed that DREADD manipulation per se or in combination with DRT induced deficits in [redacted] we will conduct the same experiments yet instead of using the DREADD+ target region viral vector, a control viral vector will be used. This in order to control for the expression of the DREADD itself on behavioural performance and the development of ICDs.

Thus, the amount of these control animals required for these experiments is dependent on the outcome of experiments described above. However, we estimate that control animals for a maximum of 3 brain pathways combined with dopamine therapy induce deficits in [redacted]

For these control groups receiving dopamine replacement therapy there are 5 Factors: brain pathway (3

pathways) X Virus (DREADD viral vectors: combination of inhibitory and excitatory with control viral vector) X dopamine replacement therapy (levodopa or pramipexole) X Dose (placebo or low dose or high dose) X cognitive task (stop-signal task, gambling task) as indicated in **Table 2**.

**PRIMARY OUTCOME PARAMETERS:** In these experiments the primary outcome measures are the effects of DREADD manipulation and dopamine therapy on response inhibition (stop-signal task) and risky decision-making (gambling task).

We will sacrifice the animals at the end of the behavioural phase of the experiment for subsequent analyses. This in order to further unravel neurobiological changes that have occurred in the specific brain pathways. We will dissect brains to perform molecular biological or neurophysiological experiments aimed at understanding the neurobiological changes that have occurred in the selected brain pathways upon DREADD manipulation and their interaction with dopamine therapy. Molecular biological experiments will focus on changes in mRNA or protein levels of various neurotransmitter receptors such as, for instance, dopamine receptors. Neurophysiological experiments will focus on functional physiological changes in the selected brain pathways that were targeted with DREADD.

**Table 2.** Estimated animal numbers in STUDY 2, DREADD + dopamine therapy:

| *STUDY 2* | *Virus injection* | *Test Drug* | *N per group* | *Total N per experiment* |
|---|---|---|---|---|
| DREADD and dopamine therapy | Combination inhibitory and excitatory DREADDs (=experimental groups) | DREADD ligand/ levodopa or pramipexole | 20 | 6 CSTC pathway X 1 DREADDs X 2 DRTs X 3 Doses X 2 Tasks X 20 rats = **1440 rats** |
| Control DREADD and dopamine therapy | Combination inhibitory and excitatory DREADDs+ control viral vector (=control groups) | DREADD ligand/ levodopa or pramipexole | 20 | **Maximal number:** 3 CSTC pathway X 1 DREADDs X 2 DRTs X 3 Doses X 2 Tasks X 20 rats = **720 rats**<br><br>**Minimal number:** 1 CSTC pathway X 1 DREADDs X 1 DRT X 3 doses X 2 Task X 20 rats = **120 rats** |

**STUDY 3. Objective 1b: Effects of adjuvant pharmacotherapy to suppress dopamine therapy-induced [redacted] deficits:**

Here to address **objective 1b**, we will assess the effects of adjuvant pharmacotherapy on levodopa- or pramipexole-induced ICDs, in the pathways that have been found to induce onset of [redacted] disturbances. We will here focus on the SSRI citalopram, the SNRI atomoxetine and the opioid antagonist naltrexone as adjuvant therapies, given 1) the beneficial effects of these compounds on [redacted] and 2) the fact that these compounds are in clinical use.

For the experimental groups receiving dopamine replacement therapy + adjuvant therapy there are 5 Factors: CSTC pathway (6 pathways) X Virus (DREADD virus: combination of inhibitory and excitatory) X

dopamine replacement therapy (levodopa or pramipexole) X Adjuvant (citalopram, atomoxetine, naltrexone) X cognitive task (stop-signal task, gambling task) as indicated in **Table 3**.

**PRIMARY OUTCOME PARAMETERS:** In these experiments the primary outcome measures are the effects of adjuvant therapy on DREADD + DRT induced deficits in response inhibition (stop-signal task) and risky decision-making (gambling task).

Similar to the ex vivo experiments described in STUDY 2, we will sacrifice the animals at the end of the behavioural phase of the experiment for subsequent analyses. This in order to further unravel neurobiological changes that have occurred in the specific brain pathways. We will dissect brains to perform molecular biological or neurophysiological experiments aimed at understanding the neurobiological changes that have occurred in the selected brain pathways upon DREADD manipulation and their interaction with dopamine therapy. Molecular biological experiments will focus on changes in mRNA or protein levels of various neurotransmitter receptors such as, for instance, dopamine receptors. Neurophysiological experiments will focus on functional physiological changes in the selected brain pathways that were targeted with DREADD.

**Table 3:** Estimated number of subjects STUDY 3, DREADD, DRT + adjuvant therapy.

| *STUDY 3* | *Virus injection* | *Test Drugs* | *N per group* | *Total N per experiment* |
|---|---|---|---|---|
| DREADD,DRT+ Adjuvant on ICD development | Combination inhibitory and excitatory DREADDs | 1.DREADD ligand/ levodopa or pramipexole<br><br>2. Adjuvant therapy: vehicle, citalopram, atomoxetine, naltrexone | 20 | **Maximal number:** 6 CSTC pathway X 1 DREADD X 1 dopamine therapy X 4 Adjuvants X 2 Task X 20 rats = **860 rats**<br><br>**Minimal number:** 1 CSTC pathway X 1 DREADD X 1 DRT X 4 Adjuvants X 2 Task X 20 rats = **160 rats** |

[1] Vardy E, et al (2015) Neuron 86: 936-946.

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

Each rat will undergo surgery, which will occur prior to the beginning of the training in the cognitive tasks. During the surgery we will provide adequate anesthesia and analgesia. After surgery we will monitor weight and general indicators of health such as presence of grooming, porphyrin secretions, etc., for a week.

Following recovery of intracranial viral surgery for DREADDs expression in the selected brain pathways, animals will be trained daily during weekdays in either the rodent stop-signal task or the rodent gambling task until stable baseline performance is achieved. This usually takes 2-3 months of task training to establish stable baseline performance. Training of these tasks takes place in operant chambers which are computer-controlled boxes containing levers, nosepoke units or other (e.g. touchscreen-operated) operanda that the subjects can operate, and visual stimuli and a pellet dispenser that can deliver highly

palatable food pellets. Both of the employed behavioural tasks are rodent analogues of existing human neuropsychological tasks, i.e. the stop-signal task and IOWA gambling task.

In all rats (with the exception of rats in STUDY 1 the pilot DREADD expression experiments) following baseline performance in the cognitive tasks, rats will receive systemic drug injections (subcutaneous or intraperitoneal) prior to a training session with the DREADD ligands to activate the inhibitory or excitatory DREADD pathway. This drug injections are fast (5 – 20 sec) and only cause mild discomfort. There is no off-target pharmacological action of the DREADD ligand, indeed this is a major advantage of the DREADD approach as the ligands only bind to the DREADD receptor. These DREADD ligand experiments will take place over a period of 2-4 weeks, with at least one day of injection-free baseline training days in between and will provide crucial information on whether inhibition or activation of a selected CSTC pathway is able to modulate [redacted]

Following these DREADD ligands tests, there will be an injection-free training period of 2-4 weeks, after which dopamine therapy (or dopamine therapy + adjuvant therapy) will commence. During dopamine therapy rats will receive a daily systemic drug injection (subcutaneous or intraperitoneal) with either placebo, levodopa or pramipexole alone (**STUDY 2, objective 1a**) or in combination with adjuvant therapy (**STUDY 3, objective 1b**; citalopram, atomoxetine, naltrexone) prior to training in the cognitive task. Dopamine therapy will last for 21 days, and on selected days during this period (e.g. day 1, 7, 14 and 21) rats will receive an additional injection with the DREADD ligand before the training session. These drug injections are fast (5 – 20 sec) and only causes mild discomfort. In STUDY 3, adjuvant therapy is given simultaneously with dopamine therapy and whenever possible combined within the same syringe and injection.

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

For statistical analysis of the primary outcome measures (response inhibition and risky decision-making), we will use analyses of variance (ANOVA) to determine significant effects between treatment groups and repeated measures ANOVA to statistically test within-subject effects. In case of statistical main effects, appropriate post-hoc comparisons will be conducted (e.g. Student-Newmann-Keuls tests, or paired T-tests).

In our experience, animal behaviour is inherently variable. There is only limited evidence available using the proposed DREADD approach combined with dopamine therapy. Therefore we conducted a power analysis taking into account recent findings employing a DREADD approach. This power analysis estimates that a group size of n=20 per group in order to have sufficient power to detect significant effects. (PARAMETERS power analysis: two-sided tests; Type I error: 5%; Power: 90%; Standard deviation: approximately 30%; Effect size: approximately 35%; Dropout rate: 20%).

In all experiments, the group sizes include the expected number of rats that will be excluded because of experimental factors, such as anatomically misplaced viral injection, rats that do not acquire stable performance in the cognitive tasks, poor health after surgery, etc. In our extensive experience the dropout rate is 20% for one of the aforementioned reasons. By allocating 20 rats per group we can reliably expect to have minimum of 16 rats included for statistical analysis, which is an appropriate number to detect statistically significant effects based on the variability of the proposed work.

**B. The animals**

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

Although we acknowledge and support the recent guideline by the National Institutes of Health (USA) to use equal numbers of male and female subjects in experimental research, we will use only male rats in the present project. This because, recent studies have indicated that estrus cycle can affect performance in cognitive tasks measuring [redacted] [1,2]. In order to control for this variability, we would need to increase the sample sizes if female rats are included. Therefore in order **to reduce** the total number of animals required for the project, the proposed work will be conducted in male rats only.

We are using rats because they have the cognitive capabilities required to understand and perform stably

in the cognitive tasks. Indeed, the rat is the best (and most used) animal to study the psychological and neural mechanisms of [redacted] Because of this it is not possible to study these questions in other species, such as for instance mice.

The rats will be approximately 12 weeks old when we receive them from the certified supplier. The experiments will typically take around 6-7 months to complete from arrival in the animal facility to end of experiment. Rats will be housed socially in pairs.

The estimated numbers are justified in **Table 1, Table 2 and Table 3** as follows:
STUDY 1: Total N=120
STUDY 2: Total N=1560 (minimum) or N=2160 (maximum)
STUDY 3: Total N=160 (minimum) or N=860 (maximum)

TOTAL estimated numbers: N=120 (minimum) or N=3140 (maximum)

[1] Diekhof (2016) Horm Behav 74: 186-193.
[2] Reimers, et al (2014) Front Neurosci 8: 401

**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.

REPLACEMENT: The rat is the best (and most widely used) animal model to study the psychological and neurobiological mechanisms of [redacted] The objectives of this project are to unravel the causal contribution of altered functioning of selected brain pathways and their interaction with dopamine therapy on the onset [redacted] disturbances. These experimental questions cannot be addressed in clinical patient populations, because we cannot directly modify functioning of these brain pathways in humans, nor combine this with dopamine therapy. Since the primary outcome measures are behavioural measures, in vitro models are not useful.

REDUCTION: Because of the variability in animal behaviour, reducing the sample size will result in much lower statistical power, potentially leading to ambiguous and non-reproducible results. The current sample sizes in the project are optimized using power analysis and the smallest possible sizes in order to obtain reliable results. Because inclusion of female rats would tremendously increase the number of animals, this project will only include male rats. Also, the inhibitory and excitatory DREADDs will be multiplexed within individuals.

REFINEMENT: We will undertake every effort to reduce the pain and suffering experienced throughout these experiments. Appropriate analgesia and anaesthetics will be used during and after the surgical procedures.
The animals will be monitored after surgery to ensure that recovery occurs as expected. All procedures that have the potential for animal harm (surgery, injections, sacrifice) will be conducted by well-trained and experienced researchers/technicians along standard operating procedures.

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

The rats will be housed in pairs and will be handled prior to training to reduce stress of initial exposure to the operant chambers. Likewise, in case of systemic injections the animals will be handled and habituated to the procedure prior to the injections.

## 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 experiments in this proposal are using novel DREADD intervention techniques in combination with novel behavioural approaches to study [redacted] deficits in rodents. These experiments have not been performed, and this specific combination of approaches is not conducted by other laboratories in the world. In consultation with colleagues in the field, we are the first to take this combined approach to develop a novel animal model mimicking Parkinson's disease.

## 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.

During the surgeries, we will use standard surgical procedure to reduce pain and suffering. Before the start of the surgery we will provide adequate pain relief. After this, we will induce anaesthesia and maintain the anaesthetised state throughout the surgery. Prior to the skin incision, we will inject (subcutaneous) a local anaesthetic into the incision site. After surgery the animal will be monitored for a

week (recording weight and general indicators of health such as presence of grooming, porphyrin secretions, etc. ), and analgesia will be administered if necessary.

**I. Other aspects compromising the welfare of the animals**

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

1. Surgeries for the viral infusions will all have an impact on the animals welfare.
2. During training in the cognitive tasks animals will be placed on a mild food restriction procedure
3. During tests with DREADD ligands and dopamine therapy (with or without adjuvant therapy) animals will receive systemic injections.

Explain why these effects may emerge.

1. Because the surgeries involve skin incision and exposing the brain (for virus injection) there is a risk of infection.
2. Apart from earning food in the cognitive tasks, rats will receive additional food daily to maintain a mild food restriction scheme. Without food-restriction, rats are not sufficiently motivated to perform in the cognitive task thereby rendering the reliability of the results. To prevent this, rats are kept on food-restriction during training in the cognitive tasks.
3. Injecting the animals will cause moderate discomfort initially that will reduce as the rat habituates to this experience, yet the discomfort remains. Unfortunately, we have no alternative to reliably deliver the DREADD ligands and dopamine/adjuvant therapy non-invasively.

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

1. Surgeries will be conducted under sterile conditions. Only when, despite these aseptic measures, infections do occur we will treat those animals with antibiotics.
2. Discomfort of drug injections will be alleviated by prior handling and habituation to the procedure. Furthermore, only trained and skilled researchers and technicians will perform the injections thereby minimizing the discomfort caused by the systemic injections. Moreover, whenever possible the different ligands will be combined within a single injection/syringe to reduce the number of injections.

**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.

We will observe the rats for the following humane endpoints throughout the experiment:

- Loss of body weight: >20% in 24 hour period.
- Immobility: If on close examination the rat is unable to move around within their homecage
- Poor coat conditions: signs that the rat is not grooming which persist for multiple days
- Tremors/Convulsions
- Self-damage
- Abnormal body posture: Any indication that the rat has suffered an injury which causes them to be unable to maintain normal body posture for an extended period of time

Indicate the likely incidence.

<2%

**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').

The total N=3140 rats that will experience the level of discomfort categorised as 'moderate'. In particular, this will occur at the start of experiments where the surgical procedure will cause moderate discomfort.

Following this animals will undergo behavioural training in the cognitive task which will cause mild discomfort paired with food-restriction which will cause moderate discomfort.

Finally, the systemic injections throughout the experiments will cause mild discomfort.

| Procedure | STUDY# | Duration | Discomfort |
|---|---|---|---|
| Surgery for DREADD virus | STUDY 1,2,3 | 1 day | Moderate |
| Food restriction | STUDY 2,3 | 5-7 months | Moderate |
| Training in cognitive tasks | STUDY 2,3 | 5-7 months | Mild |
| Systemic injections | STUDY 2,3 | 2-3 months (over this period: total expected injections per rat approximately 40-50) | Mild |
| **Cumulative discomfort** | **STUDY 1,2,3** | | **Moderate** |

## End of experiment

**L. Method of killing**

Will the animals be killed during or after the procedures?

☐ No

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

We need to analyse the rat brains with 1) immunohistochemistry to verify the expression of the viral mediated DREADDs, 2) molecular biological techniques to study ex vivo molecular changes, 3) neurophysiological techniques to study functional ex vivo physiological changes

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

# Format DEC-advies

## A. Algemene gegevens over de procedure

1. Aanvraagnummer:
*11400*

2. Titel van het project:
*Preventing* [redacted] *disorders in Parkinson's disease: a novel preclinical model*

3. Titel van de NTS:
*Het voorkomen van* [redacted] *stoornissen in de ziekte van Parkinson: een nieuw preklinisch model*

4. Type aanvraag:
*nieuwe aanvraag projectvergunning*

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

6. Adviestraject (data dd-mm-jjjj):
   - ontvangen door DEC: *31-01-2017*
   - aanvraag compleet: *31-01-2017*
   - in vergadering besproken: *14-02-2017 en 14-03-2017*
   - anderszins behandeld: *n.v.t*
   - termijnonderbreking(en) van / tot: *15-02-2017 tot 01-03-2017*
   - besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen: *n.v.t.*
   - aanpassing aanvraag: *01-03-2017*
   - advies aan CCD: *03-04-2017*

7. Geef aan of de aanvraag is afgestemd met de IvD en deze de instemming heeft van de IvD.
-Datum advies IvD: *31-01-2017*
-Strekking advies IvD: *De IvD geeft aan dat de aanvrager het project met de IvD heeft afgestemd en dat deze de instemming heeft van de IvD.*

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

9. Correspondentie met de aanvrager

*Vraagronde 1*
- Datum: *15-02-2017*
- Strekking gestelde vragen*: De inhoud van de NTS moet in overeenstemming worden gebracht met het projectvoorstel. De DEC leden hebben graag meer toelichting bij het diermodel en de treatment. De translatie van het model moet duidelijker naar voren komen, wat wordt er al gedaan in de kliniek? De uitleesparameters zijn onduidelijk, wil men het Parkinson model nabootsen of alleen het effect van de medicijnen? Men moet meer go/no go momenten benoemen. Volgens de DEC kan*

*het aantal dieren in de controle groep worden verminderd, de onderbouwing van het aantal dieren moet beter.*
- Datum antwoord: *01-03-2017*
- Strekking van de antwoord(en): *De gevraagde aanpassingen zijn doorgevoerd en de benodigde toelichting is gegeven.*
- De antwoorden hebben wel/niet geleid tot aanpassing van de aanvraag: *Ja, de antwoorden hebben geleid tot aanpassing van de aanvraag. Vanwege de aard en hoeveelheid van de vragen is de DEC van mening dat het project terug moet komen tijdens de eerstvolgende DEC vergadering op 14 maart.*

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

# B. Beoordeling (adviesvraag en behandeling)

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

# C. Beoordeling (inhoud)

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

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

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

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

   ***Belangen en waarden***

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

   *De ziekte van Parkinson is een veel voorkomende hersenaandoening die wordt gekenmerkt door ernstige motorische stoornissen veroorzaakt door tekorten in de boodschapperstof dopamine in*

*de hersenen. De behandeling van de ziekte bestaat meestal uit medicijnen die het tekort aan dopamine in de hersenen aanvullen. Echter kunnen de bijwerkingen van deze medicijnen psychiatrische afwijkingen, zogenaamde [redacted] stoornissen, veroorzaken.*

*Het directe doel van deze studie is onderzoeken welke hersengebieden verantwoordelijk kunnen zijn voor het ontstaan van [redacted] toornissen na behandeling van dopaminetekorten bij patiënten met de ziekte van Parkinson. En of het gelijktijdig gebruik van een ander medicijn een oplossing kan bieden voor het ontstaan van deze [redacted] toornissen. Het uiteindelijke doel van de studie is deze nieuw verworven kennis toe te kunnen passen voor de ontwikkeling van nieuwe therapeutische behandelingen voor de ziekte van Parkinson waarbij geen [redacted] stoornissen ontstaan.*

*Er is een reële relatie tussen deze beide doelstellingen. Het directe doel is nodig om het uiteindelijke doel in de toekomst te bereiken.*

5. Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden welke morele waarden in het geding zijn of bevorderd worden

*De belangrijkste belanghebbenden in dit project zijn: de proefdieren, de onderzoekers en de patiënten. De waarden die voor proefdieren in het geding zijn: De integriteit van de dieren zal worden aangetast omdat de dieren ingrepen ondergaan en omdat de dieren worden gedood. De waarde van deze proef voor de betrokken onderzoekers en het betreffende wetenschappelijke veld is: Het vergroten van de wetenschappelijke kennis. Waarden die voor patiënten bevorderd worden: De fundamentele kennis zal bijdragen aan het ontwikkelen van nieuwe therapeutische behandelingen voor patiënten met de ziekte van Parkinson om zo hun kwaliteit van leven te verbeteren.*

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

***Proefopzet en haalbaarheid***

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

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

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

*De aanvraag heeft een navolgbare opbouw en is naar de mening van de DEC goed opgezet. De voorgestelde experimentele opzet en uitkomstparameters zijn logisch en helder en sluiten aan bij de aangegeven doelstellingen. De DEC acht het reëel om te veronderstellen dat op basis van de resultaten van de voorgenomen reeks experimenten beschreven in het project, nieuwe en/of*

*aanvullende kennis zal worden verkregen. De nieuw verkregen inzichten kunnen bijdragen aan het beschikbaar komen van nieuwe therapeutische behandelingen voor mensen met de ziekte van Parkinson. De gevraagde looptijd van 5 jaar acht de DEC reëel gezien de opbouw, de grootte van de onderzoeksgroep en de financiële ondersteuning.*

***Welzijn dieren***

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

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

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

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

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

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

*Het cumulatieve ongerief voor alle dieren is maximaal matig. Tijdens de experimenten zal er sprake zijn van een operatie om de toediening van een virale vector mogelijk te maken en wordt voedsel-restrictie toegepast wat beide leidt tot matig ongerief. Daarnaast zullen de dieren licht ongerief ervaren als gevolg van de cognitieve gedragstaken en de systemische injecties.*

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

*De integriteit van de dieren zal worden aangetast omdat de dieren een operatie ondergaan, voedselrestrictie krijgen, gedragstaken uitvoeren, injecties krijgen en omdat de dieren worden gedood.*

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

*De criteria voor humane eindpunten zijn goed gedefinieerd. De humane eindpunten zullen worden toegepast, wanneer er duidelijke veranderingen zijn in het gewicht en het gedrag van de dieren of wanneer de dieren niet herstellen na de operatie. Men verwacht de humane eindpunten toe te passen bij minder dan 2% van de dieren. Voordat de dieren meer dan matig ongerief zullen ondervinden worden de humane eindpunten toegepast.*

***3V's***

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

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

*Hersenaandoeningen zijn zeer complex en het zeer selectief manipuleren van de hersenen om de oorzakelijke verbanden vast te stellen met betrekking tot [redacted] stoornissen en dopamine therapie is nog niet mogelijk bij mensen. Deze complexiteit is op dit moment nog niet na te bootsen met het kweken van cellen, daarom is het gebruik van proefdieren noodzakelijk. Daarnaast zullen er gedragstesten worden uitgevoerd, dit is niet mogelijk met celkweek.*

*De keuze voor het gebruik van ratten is naar het oordeel van de DEC gerechtvaardigd. De rat is de meest gebruikte diersoort in modellen voor [redacted] De gebruikte diermodellen voor [redacted] zijn direct afgeleid van neuropsychologische testen voor de mens en beschikken over een grote mate van voorspelbaarheid ten aanzien van het gedrag in mensen.*

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

*In het project wordt optimaal tegemoet gekomen aan de vereiste van de vermindering van dierproeven.*

*Door gebruik te maken van het gefaseerd uitvoeren van de experimenten en een poweranalyse wordt voorkomen dat er teveel of te weinig dieren worden gebruikt. Men zal op meerdere punten in het project een selectie maken, zo zullen vervolg experimenten alleen plaatsvinden als er een duidelijke relatie is gevonden tussen een bepaald hersengebied en [redacted] stoornissen.*

*Het maximale aantal proefdieren is proportioneel ten opzichte van de gekozen strategie en de looptijd. De DEC onderschrijft dat het project kan worden uitgevoerd met maximaal 3140 ratten en acht dit aantal realistisch onderbouwd. Onnodige duplicatie van experimenten wordt voorkomen doordat de onderzoekers goed bekend zijn met het onderzoeksveld en samenwerken met de andere onderzoeksgroepen die vergelijkbaar onderzoek verrichten.*

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

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

*De dieren krijgen tijd om te acclimatiseren, worden sociaal gehuisvest en intensief gemonitord. Passende anesthesie en pijnbestrijding zal de gevolgen van de ingrepen minimaliseren. Alle procedures zullen uitgevoerd worden door ervaren en bekwaam personeel.*

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

***Dieren in voorraad gedood en bestemming dieren na afloop proef***

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

    *In dit project zullen alleen mannelijke ratten worden gebruikt. Recente studies hebben aangegeven dat de oestrus cyclus bij vrouwelijke dieren invloed kan hebben op de prestaties van de dieren in cognitieve taken waarbij [redacted] wordt gemeten (Vriend C, et al (2014) Neurosci Biobehav Rev 38: 60-71 en Weintraub D, et al (2015) Mov Disord 30:121-127). Door mannelijke en vrouwelijke dieren te gebruiken zal de variatie binnen de groepen groter worden en zijn grotere groepen nodig om dezelfde effecten te kunnen aantonen; om deze reden is besloten om alleen mannelijke dieren te gebruiken.*

19. Geef aan of dieren gedood worden in kader van het project (tijdens of na afloop van de dierproef). Indien dieren gedood worden, geef aan of en waarom dit noodzakelijk is voor het behalen van de doelstellingen van het project. Indien dieren gedood worden, geef aan of er een voor de diersoort passende dodingsmethode gebruikt wordt die vermeld staat in bijlage IV van richtlijn 2010/63/EU. Zo niet, beoordeel of dit in voldoende mate is onderbouwd. Licht uw beoordeling toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd.

    *Na het euthanaseren van de dieren zal men het hersenweefsel verzamelen voor verdere analyse. Er wordt een dodingsmethode uit bijlage IV van richtlijn 2010/63/EU gebruikt.*

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

    ***NTS***

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

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

# D. Ethische afweging

1. Benoem de centrale morele vraag

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

    *Bij deze dierproef is de centrale morele vraag: Rechtvaardigt het verkrijgen van kennis over het ontstaan van [redacted] stoornissen na behandeling van dopaminetekorten bij de behandeling van Parkinson, en daarmee het ontwikkelen van nieuwe effectieve therapeutische behandelingen zonder [redacted] stoornissen voor patiënten met de ziekte van Parkinson het gebruik van maximaal 3140 ratten in de dierproef die daarvan maximaal matig ongerief ondervinden?*

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

*De waarden die voor de proefdieren in het geding zijn: De integriteit van de proefdieren wordt aangetast en de dieren ondervinden maximaal matig ongerief. Dat leidt tot veel nadeel voor deze proefdieren. De waarden voor de betrokken onderzoekers en het en het betreffende wetenschappelijke veld: veel voordeel vanwege de kennisontwikkeling. De waarden die voor de patiënten bevorderd worden: Mogelijk op termijn veel voordeel wanneer de dierproef bijdraagt aan het ter beschikking komen van nieuwe effectieve therapeutische behandelingen zonder [redacted] stoornissen.*

*De DEC is van mening dat de kennisontwikkeling en lange termijn belangen van de patiënten in dit project zwaarder wegen dan de belangen van de 3140 ratten die hiervoor als proefdieren gebruikt worden. Voor het verkrijgen van kennis over [redacted] stoornissen gericht op het ontwikkelen van nieuwe therapeutische behandelingen voor de ziekte van Parkinson, is onderzoek in diermodellen noodzakelijk. Er zijn op dit moment geen alternatieven voor deze dierproeven beschikbaar waarmee men de doelstellingen kan bereiken.*

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

*Volgens de DEC rechtvaardigen de doeleinden van dit project het voorgestelde gebruik van dieren. Het doel van deze studie is het verkrijgen van kennis over [redacted] stoornissen voor het ontwikkelen van nieuwe therapeutische behandelingen voor Parkinson zonder deze stoornissen als bijwerking. Het verwachte resultaat, in het kader van het beschikbaar komen van nieuwe behandelingen voor patiënten met de ziekte van Parkinson is afgewogen tegen het, als maximaal matig geschatte ongerief en de aantasting van integriteit, inclusief het doden van de dieren in de proef.*

*De DEC onderschrijft dat de doelstellingen niet zonder het gebruik van proefdieren kunnen worden behaald en acht het gebruik van maximaal 3140 ratten en de daarmee samenhangende schade aan deze dieren gerechtvaardigd. Bij het uitvoeren van de dierproeven wordt een adequate invulling gegeven aan de vereisten op het gebied van de vervanging, vermindering en verfijning van de dierproeven. Het project is (1) van substantieel belang en (2) van goede kwaliteit.*

*(1) Het wetenschappelijk en maatschappelijk belang wordt door de DEC ingeschat als substantieel. De resultaten van dit onderzoek zullen informatie geven over [redacted] stoornissen en zullen mogelijk bijdragen aan het beschikbaar komen van een effectievere behandeling van Parkinson.*

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

*Samenvattend kan worden gesteld dat het als substantieel te kwalificeren wetenschappelijk en maatschappelijk belang van het onderzoek naar het oordeel van de DEC opweegt tegen het gebruik van maximaal 3140 ratten en het daarbij verwachte matige ongerief.*

# E. Advies

1. Advies aan de CCD

*De DEC adviseert de vergunning te verlenen.*

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

*Het uitgebrachte advies is gebaseerd op consensus.*

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

*Er is geen dilemma geconstateerd.*

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Vrije Universiteit Medisch Centrum (VUmc) te
Amsterdam
T.a.v. [redacted]
[redacted] AMSTERDAM
[redacted]

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

**Onze referentie**
Aanvraagnummer
AVD1140020171289
**Bijlagen**
2

Datum 6 april 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 4 april 2017. Het gaat om uw project "Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD1140020171289. Gebruik dit nummer wanneer u contact met de CCD opneemt.

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

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

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

**Datum:**
6 april 2017
**Aanvraagnummer:**
AVD1140020171289

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

**Datum:**
6 april 2017
**Aanvraagnummer:**
AVD1140020171289

## Gegevens aanvrager

Uw gegevens

| | |
|---|---|
| Deelnemersnummer NVWA: | 11400 |
| Naam instelling of organisatie: | Vrije Universiteit Medisch Centrum (VUmc) te Amsterdam |
| Naam portefeuillehouder of diens gemachtigde: | [redacted] |
| KvK-nummer: | 64156338 |
| Straat en huisnummer: | de_Boelelaan 1117 |
| Postcode en plaats: | 1O81 HV AMSTERDAM |

Gegevens verantwoordelijke onderzoeker

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

**Datum:**
6 april 2017
**Aanvraagnummer:**
AVD1140020171289

Gegevens gemachtigde

| | |
|---|---|
| BSN: | [redacted] |
| Naam: | [redacted] |
| Adres: | [redacted] |
| Postcode en plaats: | [redacted] AMSTERDAM |
| Wilt u een nieuwe machtiging afgeven? | Ja |

**Over uw aanvraag**

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

**Over uw project**

| | |
|---|---|
| Geplande startdatum: | 1 september 2017 |
| Geplande einddatum: | 31 augustus 2022 |
| Titel project: | Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model |
| Titel niet-technische samenvatting: | Het voorkomen van [redacted] stoornissen In de ziekte van Parkinson: een nieuw preklinisch model |
| Naam DEC: | DEC Vrije Universiteit / VU Medisch Centrum |
| Postadres DEC: | [redacted]<br>1BT Amsterdam / Nederland |
| E-mailadres DEC: | [redacted] |

**Betaalgegevens**

| | |
|---|---|
| De leges bedragen: | € 1035,- |
| De leges voldoet u: | na ontvangst van de factuur |

**Checklist bijlagen**

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

**Datum:**
6 april 2017
**Aanvraagnummer:**
AVD1140020171289

**Ondertekening**

| | |
|---|---|
| Naam: | [redacted] |
| Functie: | [redacted] |
| Plaats: | Amsterdam |
| Datum: | 3 april 2017 |

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Vrije Universiteit Medisch Centrum (VUmc) te Amsterdam

[redacted]
AMSTERDAM
[redacted]

**Centrale Commissie Dierproeven**
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**Onze referentie**
Aanvraagnummer
AVD1140020171289

**Bijlagen**
2

Datum 6 april 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

**Factuur**
Factuurdatum: 6 april 2017
Vervaldatum: 6 mei 2017
Factuurnummer: 171289

| **Omschrijving** | **Bedrag** |
|---|---|
| Betaling leges projectvergunning dierproeven<br>Betreft aanvraag AVD1140020171289 | € 1035,00 |

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

7.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Vrije Universiteit Medisch Centrum (VUmc) te
Amsterdam
T.a.v. [redacted]
[redacted] AMSTERDAM
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0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

**Onze referentie**
Aanvraagnummer
AVD1140020171289

Datum 14 april 2017
Betreft aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 4 april 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model" met aanvraagnummer AVD1140020171289. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

**Welke informatie nog nodig**
Wij hebben de volgende informatie van u nodig om uw aanvraag verder te kunnen beoordelen:

**Onduidelijkheden**
1) U beschrijft milde voerrestrictie tijdens training van de cognitieve taken, wat met een matig ongerief gepaard gaat. Kunt u nader specificeren hoe deze voerrestrictie wordt uitgevoerd? (hoeveelheid voer, periodes zonder voer?).
2) Het aantal dieren dat u beschrijft in bijlage 3.4.4.1 in tabel 3 lijkt incorrect te zijn (6x1x1x4x2x20= 960 en niet 860). Kunt u dit nakijken en indien nodig aanpassen in zowel de bijlage als in de NTS?

**Leges**
De leges die u verschuldigd bent zijn nog niet door ons ontvangen of de betaling is nog niet verwerkt. Uw aanvraag is niet compleet als de leges niet zijn ontvangen.
Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

**Datum:**
14 april 2017
**Aanvraagnummer:**
AVD1140020171289

**Opsturen binnen veertien dagen**
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Stuurt u het per post op, gebruik dan het formulier dat u bij deze brief krijgt.

**Wanneer een beslissing**
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

**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,

Centrale Commissie Dierproeven

Centrale Commissie Dierproeven

# Melding bijlagen

U wilt één of meerdere bijlagen naar ons versturen? Voeg altijd deze Melding Bijlagen toe.
Wij weten dan welke documenten van u zijn en hoeveel documenten u opstuurt.
Meer informatie vindt u op www.centralecommissiedierproeven.nl
Of bel met ons: 0900 28 000 28 (10 ct/min).

**1 Uw Gegevens**

| | |
|---|---|
| Naam instelling: | Vrije Universiteit Medisch Centrum (VUmc) te Amsterdam |
| Adres: | ..... |
| Postcode en plaats: | ..... |
| Aanvraagnummer: | AVD1140020171289 |

**2 Bijlagen**
Welke bijlagen stuurt u mee?
Vink de bijlagen aan of vul de naam of omschrijving in.

O Projectvoorstel

O Beschrijving Dierproeven

O Niet-technische samenvatting

O Melding Machtiging

O Aanvraagformulier

O .....

O .....

O .....

**Datum:**
14 april 2017
**Aanvraagnummer:**
AVD1140020171289

## 3 Ondertekening

Naam: .....

Datum: ..... - ..... - .....

Handtekening: .....

Onderteken het formulier en stuur het met alle bijlagen op naar:
Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Centrale Commissie Dierproeven

# Form
## Project proposal

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

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 11400 |
| 1.2 | Provide the name of the licenced establishment. | VUmc |
| 1.3 | Provide the title of the project. | Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model |

## 2 Categories

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

- ☒ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

## 3 General description of the project

### 3.1 Background

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

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

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease. It is a progressive disorder, characterized by severe motor symptoms and motor dysfunctions and affects approximately 2% of the population. Whereas our understanding of the etiology of Parkinson's disease

has increased over the last decade, to date there is no curative therapy. One of the underlying causes of Parkinson's disease is degeneration of the brain's dopamine neurotransmitter system. Therefore in Parkinson's disease many of the motor symptoms can be improved by dopamine replacement therapy to boost the activity of the dopamine system. This dopamine replacement therapy consists of chronic treatment with the dopamine precursor levodopa and chronic treatment with dopamine receptor agonists such as pramipexole, or a combination of levodopa and dopamine receptor agonist.

Unfortunately, frequent side-effects of these dopamine replacement therapies are [redacted] disorders following onset of the treatment and they pose a major burden on patients, their families and caregivers. It is estimated that [redacted] disorders develop in 15% to even up to 25% of patients and commonly include pathological gambling, compulsive sexual behaviour, compulsive buying and binge eating [1,2]. Results of cross-sectional studies have revealed some generic factors associated with an increased risk for [redacted] disorders, e.g. young age of onset, trait impulsivity, (family)history of addiction and depression. Nonetheless, current knowledge of the characteristics related to increased susceptibility of developing [redacted] disorders is insufficient to adequately predict or prevent these disorders in daily clinical practice. More importantly, the mechanisms underlying the development and the course of [redacted] disorders in Parkinson's disease are poorly understood and cannot be directly manipulated in clinical populations.

Also, there is no effective therapy to prevent the onset of [redacted] disorders arising with dopamine therapies. Therefore having access to a translational animal model, which unravels the mechanisms and reliably predicts the risk for [redacted] disorders when treated with dopamine replacement therapy, will be a valuable model to develop novel or to refine existing pharmacotherapies for Parkinson's disease that do not induce [redacted] disorders. Moreover, the availability of such a model will also allow testing of adjuvant pharmacotherapies that alleviate [redacted] disorders.

This project aims to **develop a novel translational animal model for Parkinson's disease mimicking the risk to develop [redacted] disorders upon dopamine therapy and unraveling the underlying brain mechanisms**. As such, this novel animal model may be utilized as a preclinical tool to facilitate and aid the development of novel pharmacotherapies for Parkinson's disease that do not induce [redacted] disorders. Establishing such a model will have a great impact on the field given the sense of urgency from both clinicians as well as patients. In addition, if this novel model is successful another aim is to test whether adjuvant therapy reduces the risk to develop [redacted] disorders.

Previous and current work using different approaches to mimic Parkison's disease pathology in animals, including 6-OHDA lesions and alpha-synuclein viral overexpression, have indeed demonstrated development of [redacted] deficits and altered responsivity to dopamine therapies. However, the interventions in such animal models are irreversible, invasive by inducing permanent brain damage and lack specific or temporal control over relevant brain pathways.

To address these limitations, this project will take advantage of recent technological advances to highly selectively and temporally-precise manipulate and uncover crucial brain pathways that could drive the risk for [redacted] disorder development in Parkinson's disease. This will be achieved by the novel innovative chemogenetic approach called designer-receptors exclusively activated by designer drugs (DREADD) to mimic Parkinson-related pathology in cortico-striato-thalamo-cortical brain pathways, since these pathways are thought to underlie the risk to develop [redacted] disorders [3]. The DREADD approach will be further specified in **paragraph 3.4** (Research Strategy). First, we will combine DREADD with dopamine therapy in the same animals performing cognitive tasks that capture [redacted] deficits as a proxy to mimic clinical [redacted] disorders in Parkinson's disease. Second, upon establishing this animal model, we will study the effects of adjuvant pharmacotherapy to ameliorate dopamine therapy-induced [redacted] disorders.

[1] Vriend C, et al (2014) Neurosci Biobehav Rev 38: 60-71.
[2] Weintraub D, et al (2015) Mov Disord 30:121-127.
[3] Gerfen CR and Surmeier DJ (2011) Ann Rev Neurosci 34: 441-466.

### 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 purpose and objective** of this project is to develop a novel translational animal model that mimics the risk of developing [redacted] disorders after dopamine therapy as a preclinical animal model to facilitate and aid the development of novel pharmacotherapies without the risk of disturbed [redacted]

In line with this main objective, the specific sub-objectives are:

**Objective 1a: To establish differential effects of dopamine agonist therapy on DREADD modulation of cortico-striato-thalamo-cortical brain pathways as a proxy and proof-of-concept to mimic clinical [redacted] disorders in Parkinson's disease**

For this sub-objective, we will address the causal involvement of manipulating specific neural brain pathways in the onset of [redacted] disturbances. Furthermore, we will address the question which dopamine agonist monotherapy, levodopa or pramipexole, is most likely to induce deficits in [redacted] after subchronic treatment. Levodopa and pramipexole are different classes of dopamine therapy and clinically might possess subtle differences in the risk for onset of [redacted] disorder. Therefore, it is important to test both compounds in our novel animal model, since our model allows to further dissect the causal effects of levodopa or pramipexole treatment and their differences on deficits in [redacted]

**Objective 1b: Effects of adjuvant pharmacotherapy to suppress dopamine therapy-induced [redacted] deficits**

For this sub-objective, we will address the question whether adjuvant treatment, on top of the dopamine therapy that has the most pronounced risk to develop impulsivity, is able to suppress these effects of dopamine therapy on [redacted] deficits. More specifically, we will focus here on three different classes of clinically-used drugs as adjuvant therapies, namely 1) the antidepressant drug and selective serotonin reuptake inhibitor citalopram; 2) the anti-ADHD drug and selective norepinephrine reuptake inhibitor atomoxetine; and 3) the opioid antagonist naltrexone. These drugs are selected, since they have demonstrated to possess beneficial effects on [redacted] under different circumstances in both rats and humans, and therefore seem promising candidates as an adjuvant therapy in the clinic. However, none of these three classes of drugs have the indication Parkinson's disease, and moreover to our knowledge have not been prescribed as adjuvant therapy to dopamine replacement therapy. Therefore, our study will provide important information regarding the effectivity and applicability of this approach.

The work described in this project will be funded by an international peer-reviewed research grant from a non-profit organization that funds research into the etiology and treatment of neurodegenerative disorders including Parkinson's Disease.

The main objective and sub-objectives of the project are **achievable**, since:
1) we have ample experience using DREADD as a methodology for neural interventions that are crucial to increase our understanding of the brain pathways involved
2) we have a strong track-record and experience in translational cognitive tasks capturing [redacted] deficits, documented by many scientific papers in international journals
3) our facility has adequate numbers of operant chambers to run the cognitive tasks and housing facilities for the project
4) in addition to research technicians who will run part of the experiments, the research grant allows hiring full time staff at the postdoctoral level to complete all of these experiments within the time-frame of the project
5) all of the equipment needed for DREADD approaches is already present and the viral vectors needed are commercially available, in addition to the pharmacotherapies which are commercially available

### 3.3 Relevance

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

**Scientific relevance**
Randomized clinical trials are highly costly and lengthy in duration, therefore having access to a translational preclinical model which reliably predicts [redacted] disturbances following dopamine therapy will be a valuable model that will aid the development of novel or refinement of existing pharmacotherapies for Parkinson's disease without the risk to induce deficits in [redacted]

Furthermore, the DREADD approach of circuit-relevant and temporal specific manipulation of neural pathways will allow unique insights into the aetiology and mechanisms of [redacted] disorder development beyond the currently existing Parkinson's disease animal models and beyond what can be studied in clinical populations.

**Social relevance**
Despite the clinical efficacy of dopamine therapy in ameliorating motor symptoms in Parkinson's disease, there is no effective intervention to prevent the onset of potential deficits in [redacted] arising with pharmacotherapy. Given the tremendous burden of [redacted] disorders on patients, their families and caregivers, novel (adjuvant) pharmacotherapies that do not cause disturbances in [redacted] are highly needed. The experiments described in this project are intended to aid the development of such novel (adjuvant) pharmacotherapies for Parkinson's disease.

## 3.4 Research strategy

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

**Objective 1a: To establish differential effects of dopamine agonist therapy on DREADD modulation of cortico-striato-thalamo-cortical brain pathways as a proxy and proof-of-concept to mimic clinical [redacted] disorders in Parkinson's disease**

To address **objective 1a** and to mimic altered cortico-striato-thalamo-cortical brain pathway functioning as a way to mimic Parkinson pathology, we will employ an innovative retro-DREADD procedure [1], which permits remote control of specific neural pathways. Essentially, DREADDs (engineered G protein-coupled receptors) are targeted to specific neuronal populations using viral-vector mediated gene transfer and can be transiently activated by a synthetic pharmacological agent, that is otherwise inert. As such, DREADD provides the unique opportunity to selectively and transiently activate or silence these brain pathways and to determine their functional relevance in freely moving animals performing in cognitive tasks. This will allow unique insights into the aetiology and mechanisms of [redacted] disorder development beyond the currently existing Parkinson's disease animal models and beyond what can be studied in clinical populations.

Given the specific importance of the ventral cortico-striato-thalamo-cortical circuit in onset of [redacted] disorders, we use a dual-virus strategy to target defined neural circuits. For this, we first inject into the target region (e.g. NAc) a viral vector that is transported in a retrograde direction back to the projecting region (e.g. mPFC). In the projection region, we inject another virus that encodes the DREADD, but does not express this receptor unless the other viral vector is present. Therefore, DREADD expression is restricted to neurons defined by their circuit connectivity. With this approach, we can study the contribution of specific cortico-striato-thalamo-cortical brain pathways in mediating the effect of dopamine therapy on the onset of [redacted] deficits. The projections we aim to study are depicted in **Figure 1**, and in different groups of rats we will inject a combination of the excitatory and inhibitory DREADD virus [2] into the projection brain area and inject the other viral vector in the target region of this brain area within the same animals.

Following inhibitory or excitatory DREADD expression, animals will be trained in translational cognitive tasks including the rodent stop-signal task measuring cognitive control and a rodent gambling task measuring risk-based decision-making as a proxy of [redacted] deficits. Upon stable baseline performance, we will probe altered brain pathway functioning on cognitive control/ risk-based decisions and motor function by DREADD activation using systemic administration of DREADD ligands to either inhibit or activate the pathways under investigation. These experiments will unravel whether manipulation of specific brain pathways to mimic Parkinson pathology already induces [redacted] deficits.

Following these tests, while still trained in the cognitive tasks on a daily basis, dopamine therapy will start and animals will receive subchronic levodopa treatment or pramipexole treatment for 21 days and on days 1, 7, 14 and 21 we will additionally administer the DREADD ligands to assess the interaction between dopamine therapy and altered cortico-striato-thalamo-cortical brain pathway connectivity on

[redacted] deficits.

Based on the outcomes of these experiments we will select the specific cortico-striato-thalamo-cortical brain pathways which in combination with dopamine therapy (either levodopa or pramipexole treatment) are most likely to induce deficits in [redacted] in order to further address **objective 1b**.

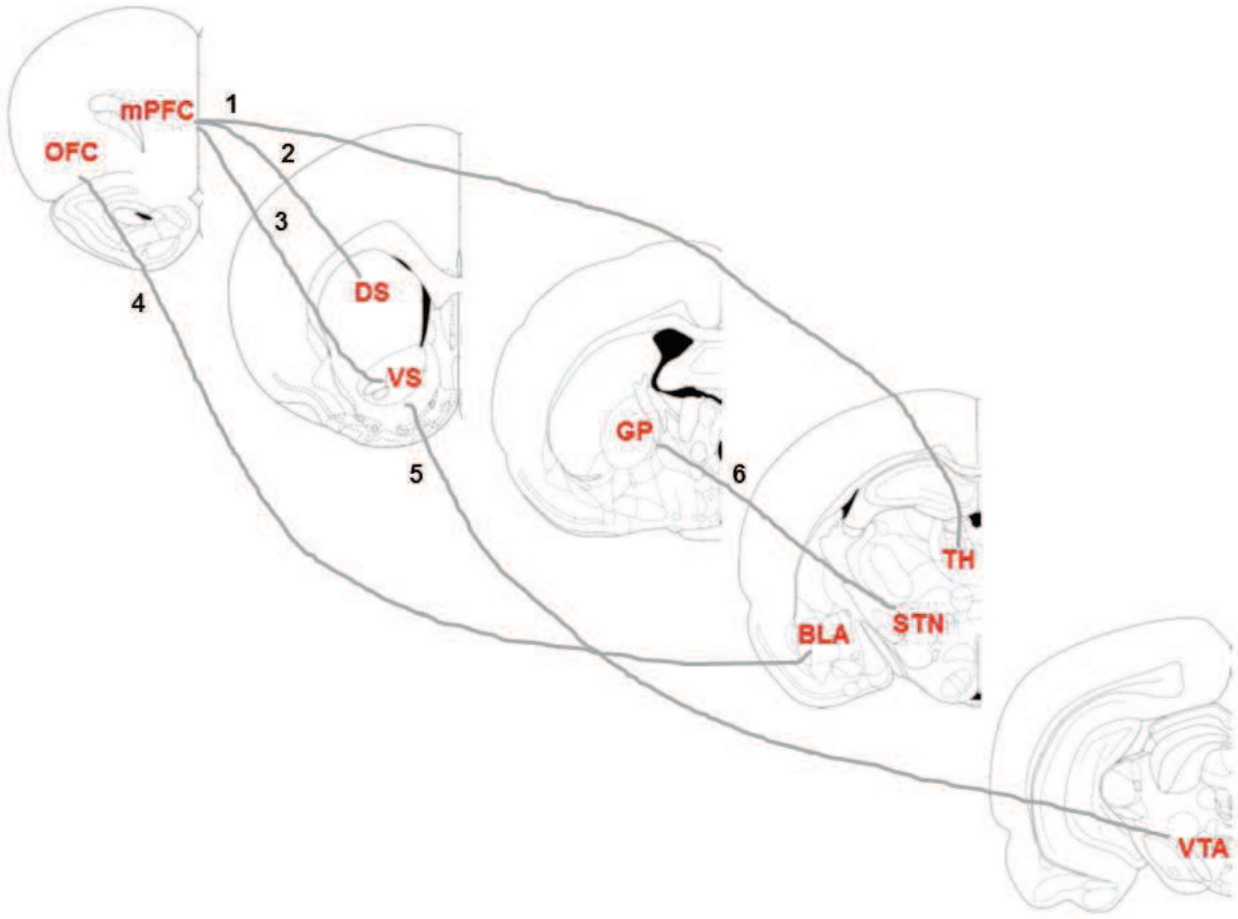


**Figure 1.** Schematic depiction of the different cortico-striato-thalamo-cortical brain pathways that will be targeted in the project. 1) thalamus to mPFC; 2) mPFC to dorsal striatum, DS; 3) mPFC to ventral striatum, VS; 4) orbitofrontal cortex, OFC, to basolateral amygdala, BLA; 5) ventral tegmental area, VTA, to VS; 6) subthalamic nucleus, STN, to globus pallidus, GP. Depicted are coronal sections of the rat brain [adapted from 3].

**Objective 1b: Effects of adjuvant pharmacotherapy to suppress dopamine therapy-induced [redacted] deficits**

In this set of experiments ,we will assess the effects of adjuvant pharmacotherapy on levodopa- or pramipexole-induced disturbances in [redacted] in the pathways that have been found to be most importantly involved in the onset of these disturbances. We will here focus on the SSRI citalopram, the SNRI atomoxetine and the opioid antagonist naltrexone as adjuvant therapies, given 1) the beneficial effects of these compounds on [redacted] and 2) the fact that these compounds are in clinical use. If successful, this will indicate that the model has immense potential for follow-up experiments to search for novel pharmacotherapies to treat Parkinson's disease without the risk of developing deficits in [redacted]

[1] Marchant NJ, et al (2016) Neuropsychopharmacology 41: 402-409.
[2] Vardy E, et al (2015) Neuron 86: 936-946.
[3] Paxinos and Watson (1998) Academic Press Ltd.

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.

As described in **paragraph 3.4** (Research Strategy), all animals in the experiments described in **both objective 1a and objective 1b** will undergo the same procedures and will be tested in each of the different components of the project following the same time-line, namely 1) viral expression of inhibitory or excitatory DREADD will be established in all animals; 2) animals will be trained in the cognitive tasks until baseline performance; 3) animals will receive pharmacological treatments with DREADD ligands and dopamine therapy (**objective 1a**) or DREADD ligands, dopamine therapy and adjuvant therapy (**objective 1b**) and 4) ex vivo analyses following behavioural testing.

Ad 1. Viral DREADDs expression:
To incorporate inhibitory or excitatory DREADDs to manipulate relevant brain pathways, viral vectors that travel along axons need to be expressed in relevant brain regions. For this, we will perform intracranial viral surgery prior to the cognitive testing. Then following recovery, animals will be trained in the cognitive tasks as described below. The primary outcome measures of viral DREADDs expression are sufficient levels of viral vector expression. This will be determined in study 1.

Ad 2. Cognitive tasks measuring [redacted] deficits:
Following recovery of the intracranial viral surgery for DREADD and viral vector expression in the selected brain pathways, animals will be trained daily during weekdays in either the rodent stop-signal task or the rodent gambling task until stable baseline performance is achieved. Training of these tasks takes place in operant chambers which are computer-controlled boxes containing levers, nosepoke units or other operanda that the subjects can operate, and visual stimuli and a pellet dispenser that can deliver small highly palatable food pellets. Both of the employed tasks are rodent analogues of existing human neuropsychological tasks, i.e. the stop-signal task and IOWA gambling task.
Briefly, in the rodent stop-signal task subjects are trained to make a response as quickly as possible once a go stimulus (=visual stimulus) is presented to earn a reward. Occasionally, this go stimulus is followed by a stop stimulus (=auditory stimulus) instructing the subject to withhold making a response. Successful inhibition upon stop stimulus presentation also leads to reward. Behavioural performance on these latter stop stimulus trials reflects response inhibition capacities and is the primary measure of the task. Poor response inhibition capacities in the task reflect [redacted] deficits.
In the rodent gambling task, that measures risky decision-making, rats are allowed to choose between 4 different response options, each one of them is associated with a different size of palatable food pellets and aversive wait periods to receive these pellets. There are 2 safe response options which are associated with low numbers of food pellets (1 or 2 pellets) and short wait periods. In contrast, the risky response options are associated with higher number of food pellets (3 or 4 pellets) and long wait periods. Optimal performance in this task would be to prefer the safe response options, which result in a nett larger win of food pellets. A preference for the risky response options results in a nett lower win of food pellets and reflects [redacted] deficits.
Primary outcome measure of these cognitive tasks is stable baseline performance in terms of response inhibition (stop-signal task) and risky decision-making (gambling task).

Ad 3. Pharmacological manipulation with DREADD ligands, dopamine therapy and adjuvant pharmacotherapy:
Upon stable baseline performance, before and during dopamine therapy we will probe altered brain pathway functioning on cognitive control/ risk-based decisions and motor function by DREADD activation using systemic administration of DREADD ligands. For subchronic dopamine therapy with either levodopa or pramipexole (**objective 1a**) or the combination of dopamine therapy with adjuvant therapy (**objective 1b**), animals will receive a single dose of the drug which will be administered peripherally once daily before testing in the cognitive tasks for 21 subsequent days. For both levodopa and pramipexole as DRT both a low and high dose will be tested in different groups using a between-subjects design. Adjuvant therapy consists of either the SSRI citalopram, the SNRI atomoxetine and the opioid antagonist naltrexone.

In these experiments, the primary outcome measures are the effects of DREADD manipulation and dopamine therapy alone or combined with adjuvant pharmacotherapy on response inhibition (stop-signal task) and risky decision-making (gambling task).

Ad 4. Ex vivo analyses
We will sacrifice the animals at the end of the behavioural phase of experiments for subsequent ex vivo analyses. This in order to further unravel neurobiological changes that have occurred in the specific brain pathways. We will dissect brains to perform molecular biological or neurophysiological experiments aimed at understanding the neurobiological changes that have occurred in the selected brain pathways upon DREADD manipulation and their interaction with dopamine therapy. Molecular biological experiments will focus on changes in mRNA or protein levels of various neurotransmitter receptors such as, for instance, dopamine receptors as primary outcome measures. Neurophysiological experiments will focus on functional physiological changes in the selected brain pathways that were targeted with DREADD as primary outcome measures.

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.

The coherence between the different components and steps of the project is depicted in **Figure 2**.

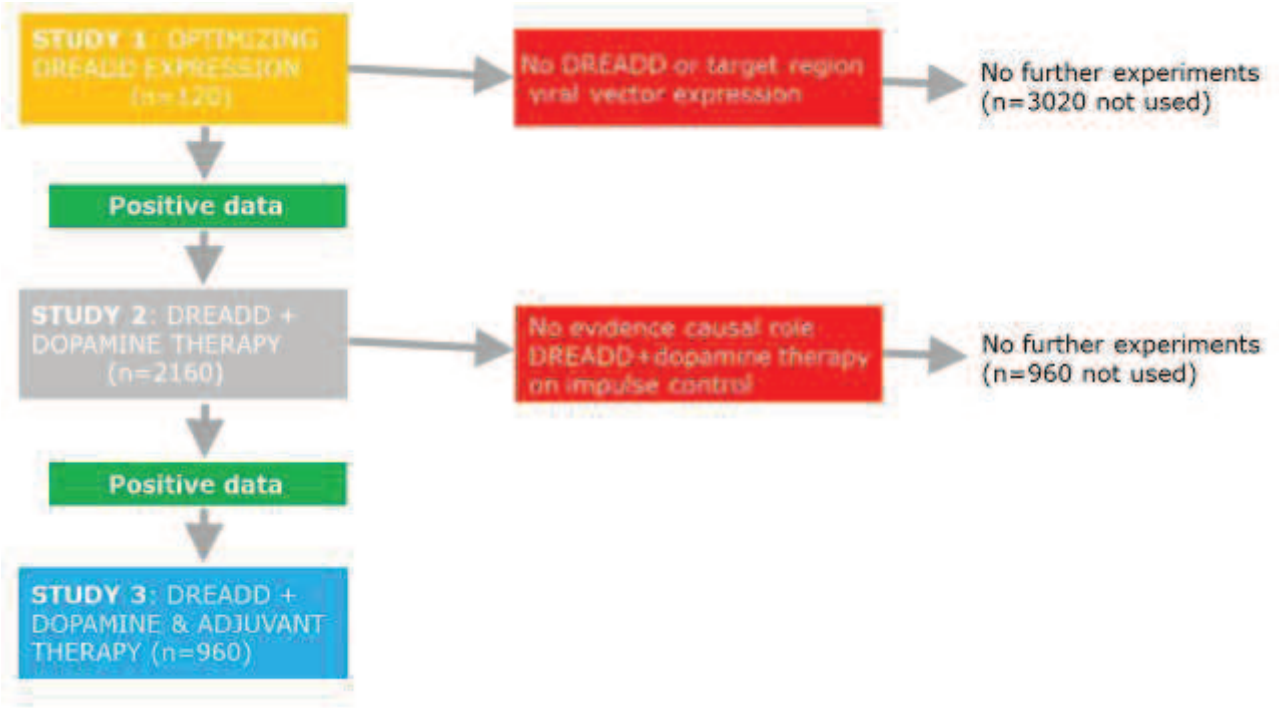


***Figure 2.*** *Outline of decision points where animals will be used or not depending on the outcome of experiments. Note that only the maximum estimated numbers of animals within each study are depicted.*

**STUDY 1 (Milestone 1):**
In order to optimize the expression levels of the inhibitory or excitatory DREADD virus and viral vector in the target region, we will first conduct pilot studies, in which we intracranially express these viruses. These studies we will determine the optimal conditions for DREADD expression and viral vector expression in the different cortico-striato-thalamo-cortical pathways.

**STUDY 2 (Milestone 2):**
To address **objective 1a**, we will combine inhibitory and excitatory DREADDs in different brain pathways

with either levodopa or pramipexole to develop a novel animal model to study deficits in [redacted] in Parkinson's disease. Thus this novel model aims to elucidate altered brain pathways that combined with dopamine therapy induce disturbed [redacted] as a proxy of [redacted] disorders in Parkinson's disease. Therefore, we will perform experiments described under this objective to pinpoint which combination of brain pathway DREADD manipulation and dopamine therapy is most powerful to evoke and to mimic [redacted] deficits.

**STUDY 3 (Milestone 3):**

To address **objective 1b**, we will test whether adjuvant pharmacotherapy (citalopram, atomoxetine and naltrexone) is capable of suppressing and preventing the onset of deficits in [redacted] after dopamine therapy. Here we will only study the DREADD brain pathways and dopamine therapy combinations that have induced [redacted] deficits. As such, the number of experiments and subjects required here depends on the results of the second series. We will test all three different adjuvant pharmacotherapies in these selected relevant DREADD brain pathways and dopamine therapy combinations.

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 | DREADD expression and pharmacotherapeutic interventions during behaviour |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 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.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 11400 |
| 1.2 | Provide the name of the licenced establishment. | VUmc |
| 1.3 | List the serial number and type of animal procedure.<br><br>*Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.* | Serial number: 1<br>Type of animal procedure: DREADD expression and pharmacotherapeutic interventions during behaviour |

## 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.

The general outline of the experiments of the project is depicted below in **Figure 1**.

**Figure 1.** General outline of experiments. **STUDY 1:** Pilot studies for optimizing DREADD expression in selected brain pathways. **STUDY 2:** DREADD in selected brain pathways combined with dopamine therapy alone to study onset of deficits in [redacted] **STUDY 3:** DREADD in selected brain pathways combined with dopamine therapy + adjuvant therapy to ameliorate deficits in [redacted]

**STUDY 1. Pilot studies to optimize DREADD expression in selected brain pathways**

In order to develop a novel animal model mimicking the pathology of Parkinson's disease, we use a dual-virus strategy to target specific cortico-striato-thalamo-cortical brain pathways. For this, we first inject into the target region (e.g. NAc) a viral vector that is transported in a retrograde direction back to the projecting region (e.g. mPFC). In the projection region, we inject a virus that encodes the DREADD, but does not express this receptor the viral vector is present. Therefore, DREADD expression is restricted to neurons defined by their circuit connectivity. With this approach, we can study the contribution of different brain pathways in mediating the effect of DRT on ICD development.

Prior to conducting the behavioural experiments, we will optimize the viral expression patterns of the DREADD constructs in each of the selected brain pathways in pilot studies before we proceed with this pathway in the behavioural experiments. As shown in **Table 1**, for these pilot studies there are 2 Factors: brain pathway (6 pathways) X Virus (DREADD virus: inhibitory or excitatory).

During two surgeries, we will place the rat into the stereotaxic frame and use a needle attached to the stereotact to accurately inject the two viral vectors (DREADD and target region viral vector) into the specific brain regions (projection and target region). After recovery and a waiting period of approximately 4-8 weeks to allow sufficient DREADD expression, animals will be sacrificed to determine viral expression patterns in brain tissue.

**PRIMARY OUTCOME PARAMETERS:** The primary outcome of these pilot studies is the level of DREADD expression and target region viral vector expression in the selected brain pathways. These expression levels should be sufficient in order to continue with the behavioural experiments.

**Table 1.** Estimated animal numbers in STUDY 1, pilots for DREADD expression

| *STUDY 1* | *Virus injection* | *Test Drug* | *N per group* | *Total N per experiment* |
|---|---|---|---|---|
| Pilot infusions DREADD in selected brain pathways for sufficient expression levels | Inhibitory and excitatory DREADDs +target region viral vector | none | 10 | 2 DREADDs X<br>6 CSTC pathways X<br>10 rats =<br>**120 rats** |

**STUDY 2. Objective 1a: Differential effects of dopamine agonist therapy on DREADD modulation of cortico-striato-thalamo-cortical brain pathways as a proxy and proof-of-concept to mimic clinical [redacted] disorders in Parkinson's disease**

Here we will combine DREADD in different cortico-striato-thalamo-cortical circuits with either levodopa or pramipexole dopamine therapy in animals that are trained in the stop-signal task to measure response inhibition, or a gambling task to measure risky decision-making. This in order to unravel the underlying brain pathways and mechanisms as a novel model to mimic [redacted] disorders in Parkinson's disease.

Description of the cognitive tasks:
In Parkinson's disease, deficits in [redacted] are characterized by an inability to suppress certain (inappropriate) impulses that may lead to maladaptive behaviour including hypersexuality, compulsive

buying and gambling behaviour. To capitulate on these different clinical aspects using a translational approach we will employ 1) the stop-signal task to measure cognitive control and 2) the rodent gambling task to measure risky decision making. These tasks are instrumental learning tasks which take place in operant chambers outside of the home cage of the animals. Training in each of these tasks takes 2-3 months daily training per task and since both tasks measure different forms of [redacted] it is not feasible to test both tasks within the same individuals. Therefore separate groups of animals will be required for each task.

Ad 1) In laboratory settings response inhibition is an often used behavioural measure to assess cognitive control, that is the ability to control one's impulses. The stop-signal task measures response inhibition and requires subjects to inhibit inappropriate responses when certain cues, in this case stop-signals are provided. Briefly, in this task subjects are trained to make a response as quickly as possible once a go stimulus (=visual stimulus) is presented. Occasionally, this go stimulus is followed by a stop stimulus (=auditory stimulus) instructing the subject to withhold making a response. Behavioural performance on these latter stop stimulus trials reflects response inhibition capacities and is the primary measure of the task. Whereas the stop-signal task is designed to primarily tax response inhibition, the task also measures reaction latencies on go trials, prematurely expressed responses before go stimulus presentations and omitted trials. These parameters control for other behavioural alterations such as motivation to participate in the task (omitted trials), general deficits in inhibitory control (prematurely expressed responses) and changes in motor performance (reaction latencies and omitted trials).

Ad 2) The rodent gambling task will be employed to assess risky-decision making. This translational task is adopted from the Iowa gambling task and requires rats to choose between 4 different response options, each one of them is associated with a different size of palatable food pellets and aversive wait periods to receive these pellets. The safe response options are associated with low numbers of food pellets (1 or 2 pellets) and short wait periods, whereas the risky options are associated with higher number of food pellets (3 or 4 pellets) and long wait periods. Optimal performance in this task would be to prefer the safe response options, which result in a nett larger win of food pellets. A preference for the risky response options results in a nett lower win of food pellets and reflects [redacted] deficits.

Estimated number of animals

For the experimental groups receiving dopamine replacement therapy there are 5 Factors: brain pathway (6 pathways) X Virus (DREADD viral vectors: combination of inhibitory and excitatory) X dopamine replacement therapy (levodopa, pramipexole) X Dose (placebo, low dose, high dose) X cognitive task (stop-signal task, gambling task) as indicated in **Table 2**.

During surgery, we will place the rat into the stereotaxic frame and use a needle attached to the stereotact to accurately inject the two viruses (DREADD and target region viral vector) into the specific brain regions (projection and target region). Based on recent work showing successful combination of excitatory and inhibitory DREADDs by multiplexing these different DREADDs within individuals [1], we will also multiplex these DREADDs within the same individuals. This will lead to a tremendous **reduction** in the number of animals used in the project. After recovery, animals will be trained daily in the cognitive tasks and upon establishment of baseline performance, first tests with the DREADD ligands will be conducted in the cognitive tasks, followed by dopamine therapy in combination with DREADD ligands in the cognitive tasks.

*Control DREADD virus experiments:*

Only in the CSTC pathways in which we observed that DREADD manipulation per se or in combination with DRT induced deficits in [redacted] we will conduct the same experiments yet instead of using the DREADD+ target region viral vector, a control viral vector will be used. This in order to control for the expression of the DREADD itself on behavioural performance and the development of ICDs.

Thus, the amount of these control animals required for these experiments is dependent on the outcome of experiments described above. However, we estimate that control animals for a maximum of 3 brain pathways combined with dopamine therapy induce deficits in [redacted]

For these control groups receiving dopamine replacement therapy there are 5 Factors: brain pathway (3

pathways) X Virus (DREADD viral vectors: combination of inhibitory and excitatory with control viral vector) X dopamine replacement therapy (levodopa or pramipexole) X Dose (placebo or low dose or high dose) X cognitive task (stop-signal task, gambling task) as indicated in **Table 2**.

**PRIMARY OUTCOME PARAMETERS:** In these experiments the primary outcome measures are the effects of DREADD manipulation and dopamine therapy on response inhibition (stop-signal task) and risky decision-making (gambling task).

We will sacrifice the animals at the end of the behavioural phase of the experiment for subsequent analyses. This in order to further unravel neurobiological changes that have occurred in the specific brain pathways. We will dissect brains to perform molecular biological or neurophysiological experiments aimed at understanding the neurobiological changes that have occurred in the selected brain pathways upon DREADD manipulation and their interaction with dopamine therapy. Molecular biological experiments will focus on changes in mRNA or protein levels of various neurotransmitter receptors such as, for instance, dopamine receptors. Neurophysiological experiments will focus on functional physiological changes in the selected brain pathways that were targeted with DREADD.

**Table 2.** Estimated animal numbers in STUDY 2, DREADD + dopamine therapy:

| *STUDY 2* | *Virus injection* | *Test Drug* | *N per group* | *Total N per experiment* |
|---|---|---|---|---|
| DREADD and dopamine therapy | Combination inhibitory and excitatory DREADDs (=experimental groups) | DREADD ligand/ levodopa or pramipexole | 20 | 6 CSTC pathway X 1 DREADDs X 2 DRTs X 3 Doses X 2 Tasks X 20 rats = **1440 rats** |
| Control DREADD and dopamine therapy | Combination inhibitory and excitatory DREADDs+ control viral vector (=control groups) | DREADD ligand/ levodopa or pramipexole | 20 | **Maximal number:** 3 CSTC pathway X 1 DREADDs X 2 DRTs X 3 Doses X 2 Tasks X 20 rats = **720 rats**<br><br>**Minimal number:** 1 CSTC pathway X 1 DREADDs X 1 DRT X 3 doses X 2 Task X 20 rats = **120 rats** |

**STUDY 3. Objective 1b: Effects of adjuvant pharmacotherapy to suppress dopamine therapy-induced [redacted] deficits:**

Here to address **objective 1b**, we will assess the effects of adjuvant pharmacotherapy on levodopa- or pramipexole-induced ICDs, in the pathways that have been found to induce onset of [redacted] disturbances. We will here focus on the SSRI citalopram, the SNRI atomoxetine and the opioid antagonist naltrexone as adjuvant therapies, given 1) the beneficial effects of these compounds on [redacted] and 2) the fact that these compounds are in clinical use.

For the experimental groups receiving dopamine replacement therapy + adjuvant therapy there are 5 Factors: CSTC pathway (6 pathways) X Virus (DREADD virus: combination of inhibitory and excitatory) X

dopamine replacement therapy (levodopa or pramipexole) X Adjuvant (citalopram, atomoxetine, naltrexone) X cognitive task (stop-signal task, gambling task) as indicated in **Table 3**.

**PRIMARY OUTCOME PARAMETERS:** In these experiments the primary outcome measures are the effects of adjuvant therapy on DREADD + DRT induced deficits in response inhibition (stop-signal task) and risky decision-making (gambling task).

Similar to the ex vivo experiments described in STUDY 2, we will sacrifice the animals at the end of the behavioural phase of the experiment for subsequent analyses. This in order to further unravel neurobiological changes that have occurred in the specific brain pathways. We will dissect brains to perform molecular biological or neurophysiological experiments aimed at understanding the neurobiological changes that have occurred in the selected brain pathways upon DREADD manipulation and their interaction with dopamine therapy. Molecular biological experiments will focus on changes in mRNA or protein levels of various neurotransmitter receptors such as, for instance, dopamine receptors. Neurophysiological experiments will focus on functional physiological changes in the selected brain pathways that were targeted with DREADD.

**Table 3:** Estimated number of subjects STUDY 3, DREADD, DRT + adjuvant therapy.

| *STUDY 3* | *Virus injection* | *Test Drugs* | *N per group* | *Total N per experiment* |
|---|---|---|---|---|
| DREADD,DRT+ Adjuvant on ICD development | Combination inhibitory and excitatory DREADDs | 1.DREADD ligand/ levodopa or pramipexole<br><br>2. Adjuvant therapy: vehicle, citalopram, atomoxetine, naltrexone | 20 | **Maximal number:** 6 CSTC pathway X 1 DREADD X 1 dopamine therapy X 4 Adjuvants X 2 Task X 20 rats = **960 rats**<br><br>**Minimal number:** 1 CSTC pathway X 1 DREADD X 1 DRT X 4 Adjuvants X 2 Task X 20 rats = **160 rats** |

[1] Vardy E, et al (2015) Neuron 86: 936-946.

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

Each rat will undergo surgery, which will occur prior to the beginning of the training in the cognitive tasks. During the surgery we will provide adequate anesthesia and analgesia. After surgery we will monitor weight and general indicators of health such as presence of grooming, porphyrin secretions, etc., for a week.

Following recovery of intracranial viral surgery for DREADDs expression in the selected brain pathways, animals will be trained daily during weekdays in either the rodent stop-signal task or the rodent gambling task until stable baseline performance is achieved. This usually takes 2-3 months of task training to establish stable baseline performance. Training of these tasks takes place in operant chambers which are computer-controlled boxes containing levers, nosepoke units or other (e.g. touchscreen-operated) operanda that the subjects can operate, and visual stimuli and a pellet dispenser that can deliver highly

palatable food pellets. Both of the employed behavioural tasks are rodent analogues of existing human neuropsychological tasks, i.e. the stop-signal task and IOWA gambling task.

In all rats (with the exception of rats in STUDY 1 the pilot DREADD expression experiments) following baseline performance in the cognitive tasks, rats will receive systemic drug injections (subcutaneous or intraperitoneal) prior to a training session with the DREADD ligands to activate the inhibitory or excitatory DREADD pathway. This drug injections are fast (5 – 20 sec) and only cause mild discomfort. There is no off-target pharmacological action of the DREADD ligand, indeed this is a major advantage of the DREADD approach as the ligands only bind to the DREADD receptor. These DREADD ligand experiments will take place over a period of 2-4 weeks, with at least one day of injection-free baseline training days in between and will provide crucial information on whether inhibition or activation of a selected CSTC pathway is able to modulate [redacted]

Following these DREADD ligands tests, there will be an injection-free training period of 2-4 weeks, after which dopamine therapy (or dopamine therapy + adjuvant therapy) will commence. During dopamine therapy rats will receive a daily systemic drug injection (subcutaneous or intraperitoneal) with either placebo, levodopa or pramipexole alone (**STUDY 2, objective 1a**) or in combination with adjuvant therapy (**STUDY 3, objective 1b**; citalopram, atomoxetine, naltrexone) prior to training in the cognitive task. Dopamine therapy will last for 21 days, and on selected days during this period (e.g. day 1, 7, 14 and 21) rats will receive an additional injection with the DREADD ligand before the training session. These drug injections are fast (5 – 20 sec) and only causes mild discomfort. In STUDY 3, adjuvant therapy is given simultaneously with dopamine therapy and whenever possible combined within the same syringe and injection.

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

For statistical analysis of the primary outcome measures (response inhibition and risky decision-making), we will use analyses of variance (ANOVA) to determine significant effects between treatment groups and repeated measures ANOVA to statistically test within-subject effects. In case of statistical main effects, appropriate post-hoc comparisons will be conducted (e.g. Student-Newmann-Keuls tests, or paired T-tests).

In our experience, animal behaviour is inherently variable. There is only limited evidence available using the proposed DREADD approach combined with dopamine therapy. Therefore we conducted a power analysis taking into account recent findings employing a DREADD approach. This power analysis estimates that a group size of n=20 per group in order to have sufficient power to detect significant effects. (PARAMETERS power analysis: two-sided tests; Type I error: 5%; Power: 90%; Standard deviation: approximately 30%; Effect size: approximately 35%; Dropout rate: 20%).

In all experiments, the group sizes include the expected number of rats that will be excluded because of experimental factors, such as anatomically misplaced viral injection, rats that do not acquire stable performance in the cognitive tasks, poor health after surgery, etc. In our extensive experience the dropout rate is 20% for one of the aforementioned reasons. By allocating 20 rats per group we can reliably expect to have minimum of 16 rats included for statistical analysis, which is an appropriate number to detect statistically significant effects based on the variability of the proposed work.

**B. The animals**

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

Although we acknowledge and support the recent guideline by the National Institutes of Health (USA) to use equal numbers of male and female subjects in experimental research, we will use only male rats in the present project. This because, recent studies have indicated that estrus cycle can affect performance in cognitive tasks measuring [redacted] [1,2]. In order to control for this variability, we would need to increase the sample sizes if female rats are included. Therefore in order **to reduce** the total number of animals required for the project, the proposed work will be conducted in male rats only.

We are using rats because they have the cognitive capabilities required to understand and perform stably

in the cognitive tasks. Indeed, the rat is the best (and most used) animal to study the psychological and neural mechanisms of [redacted] Because of this it is not possible to study these questions in other species, such as for instance mice.

The rats will be approximately 12 weeks old when we receive them from the certified supplier. The experiments will typically take around 6-7 months to complete from arrival in the animal facility to end of experiment. Rats will be housed socially in pairs.

The estimated numbers are justified in **Table 1, Table 2 and Table 3** as follows:
STUDY 1: Total N=120
STUDY 2: Total N=1560 (minimum) or N=2160 (maximum)
STUDY 3: Total N=160 (minimum) or N=960 (maximum)

TOTAL estimated numbers: N=120 (minimum) or N=3240 (maximum)

[1] Diekhof (2016) Horm Behav 74: 186-193.
[2] Reimers, et al (2014) Front Neurosci 8: 401

**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.

REPLACEMENT: The rat is the best (and most widely used) animal model to study the psychological and neurobiological mechanisms of [redacted] The objectives of this project are to unravel the causal contribution of altered functioning of selected brain pathways and their interaction with dopamine therapy on the onset [redacted] disturbances. These experimental questions cannot be addressed in clinical patient populations, because we cannot directly modify functioning of these brain pathways in humans, nor combine this with dopamine therapy. Since the primary outcome measures are behavioural measures, in vitro models are not useful.

REDUCTION: Because of the variability in animal behaviour, reducing the sample size will result in much lower statistical power, potentially leading to ambiguous and non-reproducible results. The current sample sizes in the project are optimized using power analysis and the smallest possible sizes in order to obtain reliable results. Because inclusion of female rats would tremendously increase the number of animals, this project will only include male rats. Also, the inhibitory and excitatory DREADDs will be multiplexed within individuals.

REFINEMENT: We will undertake every effort to reduce the pain and suffering experienced throughout these experiments. Appropriate analgesia and anaesthetics will be used during and after the surgical procedures.
The animals will be monitored after surgery to ensure that recovery occurs as expected. All procedures that have the potential for animal harm (surgery, injections, sacrifice) will be conducted by well-trained and experienced researchers/technicians along standard operating procedures.

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

The rats will be housed in pairs and will be handled prior to training to reduce stress of initial exposure to the operant chambers. Likewise, in case of systemic injections the animals will be handled and habituated to the procedure prior to the injections.

## 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 experiments in this proposal are using novel DREADD intervention techniques in combination with novel behavioural approaches to study [redacted] deficits in rodents. These experiments have not been performed, and this specific combination of approaches is not conducted by other laboratories in the world. In consultation with colleagues in the field, we are the first to take this combined approach to develop a novel animal model mimicking Parkinson's disease.

## 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.

During the surgeries, we will use standard surgical procedure to reduce pain and suffering. Before the start of the surgery we will provide adequate pain relief. After this, we will induce anaesthesia and maintain the anaesthetised state throughout the surgery. Prior to the skin incision, we will inject (subcutaneous) a local anaesthetic into the incision site. After surgery the animal will be monitored for a

week (recording weight and general indicators of health such as presence of grooming, porphyrin secretions, etc. ), and analgesia will be administered if necessary.

**I. Other aspects compromising the welfare of the animals**

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

1. Surgeries for the viral infusions will all have an impact on the animals welfare.
2. During training in the cognitive tasks animals will be placed on a mild food restriction procedure to maintain their weight at approximately 85% of their free-feeding weight.
3. During tests with DREADD ligands and dopamine therapy (with or without adjuvant therapy) animals will receive systemic injections.

Explain why these effects may emerge.

1. Because the surgeries involve skin incision and exposing the brain (for virus injection) there is a risk of infection.
2. Apart from earning food in the cognitive tasks, rats will receive additional food daily to maintain a mild food restriction scheme and maintain rats at approximately 85% of their free-feeding weight. Without food-restriction, rats are not sufficiently motivated to perform in the cognitive task thereby rendering the reliability of the results. To prevent this, rats are kept on food-restriction during training in the cognitive tasks and receive additional food every day.
3. Injecting the animals will cause moderate discomfort initially that will reduce as the rat habituates to this experience, yet the discomfort remains. Unfortunately, we have no alternative to reliably deliver the DREADD ligands and dopamine/adjuvant therapy non-invasively.

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

1. Surgeries will be conducted under sterile conditions. Only when, despite these aseptic measures, infections do occur we will treat those animals with antibiotics.
2. Discomfort of drug injections will be alleviated by prior handling and habituation to the procedure. Furthermore, only trained and skilled researchers and technicians will perform the injections thereby minimizing the discomfort caused by the systemic injections. Moreover, whenever possible the different ligands will be combined within a single injection/syringe to reduce the number of injections.

**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.

We will observe the rats for the following humane endpoints throughout the experiment:

- Loss of body weight: >20% in 24 hour period.
- Immobility: If on close examination the rat is unable to move around within their homecage
- Poor coat conditions: signs that the rat is not grooming which persist for multiple days
- Tremors/Convulsions
- Self-damage
- Abnormal body posture: Any indication that the rat has suffered an injury which causes them to be unable to maintain normal body posture for an extended period of time

Indicate the likely incidence.

<2%

**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').

The total N=3240 rats that will experience the level of discomfort categorised as 'moderate'. In particular, this will occur at the start of experiments where the surgical procedure will cause moderate discomfort.

Following this animals will undergo behavioural training in the cognitive task which will cause mild discomfort paired with food-restriction which will cause moderate discomfort.

Finally, the systemic injections throughout the experiments will cause mild discomfort.

| Procedure | STUDY# | Duration | Discomfort |
|---|---|---|---|
| Surgery for DREADD virus | STUDY 1,2,3 | 1 day | Moderate |
| Food restriction to approximately 85% of free-feeding weight | STUDY 2,3 | 5-7 months | Moderate |
| Training in cognitive tasks | STUDY 2,3 | 5-7 months | Mild |
| Systemic injections | STUDY 2,3 | 2-3 months (over this period: total expected injections per rat approximately 40-50) | Mild |
| **Cumulative discomfort** | **STUDY 1,2,3** | | **Moderate** |

## End of experiment

**L. Method of killing**

Will the animals be killed during or after the procedures?

☐ No

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

We need to analyse the rat brains with 1) immunohistochemistry to verify the expression of the viral mediated DREADDs, 2) molecular biological techniques to study ex vivo molecular changes, 3) neurophysiological techniques to study functional ex vivo physiological changes

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

12.

Centrale Commissie Dierproeven

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AVD1140020171289
**Bijlagen**
1

26 APR. 2017

Datum 25 april 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 3 april 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model" met aanvraagnummer AVD1140020171289. Wij hebben uw aanvraag beoordeeld.

Op 20 april 2017 heeft u uw aanvraag aangevuld. Het voerrestrictie regime is nader beschreven en een miscalculatie in dieraantallen is verbeterd.

**Beslissing**
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
De algemene voorwaarden zijn opgenomen op grond van artikel 1d lid 4, artikel 10a1 lid 2, artikel 10 lid 2 en/of artikel 10a3 van de wet.

U kunt met uw project "Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model" starten. De vergunning wordt afgegeven van 1 september 2017 tot en met 31 augustus 2022.

Overige wettelijke bepalingen blijven van kracht.

**Procedure**
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC Vrije Universiteit / VU Medisch Centrum gevoegd. Dit advies is opgesteld op 3 april 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 24 april 2017 heeft de DEC gereageerd

op onze vragen. De communicatie met de onderzoeker is weergegeven. Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

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

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

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

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

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

**Datum:**
25 april 2017
**Aanvraagnummer:**
AVD1140020171289

Centrale Commissie Dierproeven
namens deze:

[redacted]

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

Centrale Commissie Dierproeven

# Projectvergunning

**gelet op artikel 10a van de Wet op de Dierproeven**

Verleent de Centrale Commissie Dierproeven aan

| | |
|---|---|
| Naam: | Vrije Universiteit Medisch Centrum (VUmc) te Amsterdam |
| Adres: | de_Boelelaan 1117 |
| Postcode en plaats: | 1O81 HV AMSTERDAM |
| Deelnemersnummer: | 11400 |

deze projectvergunning voor het tijdvak 1 september 2017 tot en met 31 augustus 2022, voor het project "Preventing [redacted] disorders in Parkinson's disease: a novel preclinical model" met aanvraagnummer AVD1140020171289, volgens advies van Dierexperimentencommissie DEC Vrije Universiteit / VU Medisch Centrum. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is [redacted]
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 3 april 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 20 april 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 20 april 2017;
c Advies van dierexperimentencommissie d.d. 3 april 2017, ontvangen op 3 april 2017.
d De aanvullingen op uw aanvraag, ontvangen op 20 april 2017

| Naam proef | Diersoort/ Stam | Aantal dieren | Ernst | Opmerkingen |
|---|---|---|---|---|
| **3.4.4.1 DREADD expression and pharmacotherapeutic interventions during behaviour** | | | | |
| | Ratten (Rattus norvegicus) / | 3.240 | 100% Matig | |

**Voorwaarden**

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

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

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is.

**Aanvraagnummer:**
AVD1140020171289

Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Centrale Commissie Dierproeven

**Aanvraagnummer:**
AVD1140020171289

# Weergave wet- en regelgeving

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

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

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

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

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