	Inventaris Wob-verzoek W16-21S								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS2016543								
1	Aanvraagformulier				x		x		
2	Projectvoorstel			x					
3	Niet-technische samenvatting oud			x					
4	Bijlage beschrijving dierproeven 1			x					
5	Bijlage beschrijving dierproeven 1			x					
6	DEC-advies				x		x		
7	Ontvangstbevestiging				x		x		
8	Verzoek aanvulling aanvraag				x		x		
9	Niet technische samenvatting herzien	x		x					
10	Adviesnota CCD		x						x
11	Beschikking en vergunning				x		x		

Centrale Commissie Dierproeven

1.

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

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

1 Gegevens aanvrager

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

☒ Ja > *Vul uw deelnemernummer in* 10700
☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	Universiteit Maastricht
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	50169181

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

Straat en huisnummer	Minderbroedersberg	4-6
Postbus	616	
Postcode en plaats	6200 MD	Maastricht
IBAN	NL04 INGB 0679 5101 68	
Tenaamstelling van het rekeningnummer	Universiteit Maastricht	

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	[redacted]	☒ Dhr. ☐ Mw.
Functie	[redacted]	
Afdeling	[redacted]	
Telefoonnummer	[redacted]	
E-mailadres	[redacted]	

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

2 Over uw aanvraag

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

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 3 - 2016
		Einddatum	1 - 3 - 2021
3.2	Wat is de titel van het project?	Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease	
3.3	Wat is de titel van de niet-technische samenvatting?	Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC UM
		Postadres	Postbus 616, 6200MD Maastricht
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1441,00 Lege

☐ Wijziging € Lege

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

☐ Via een eenmalige incasso

☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☒

6 Ondertekening

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

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

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

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

Naam	[redacted]
Functie	
Plaats	Maastri
Datum	9 - 5
Handtekening	

Centrale Commissie Dierproeven

2.

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'.	10700
1.2	Provide the name of the licenced establishment.	University Maastricht
1.3	Provide the title of the project.	Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease

2 Categories

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

- X 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.

Deep brain stimulation (DBS) involves the implantation of stimulating electrodes into specific parts of the brain. DBS is a rapidly emerging area of clinical neuroscience and has evolved to be an effective treatment for patients with Parkinson's disease (PD) [6], essential tremor [7-8], and dystonia [9]. In

patients with severe Tourette syndrome [10] and Obsessive Compulsive Disorder [11-12], DBS can produce therapeutic effects as well. In other neurological and psychiatric disorders, such as Huntington's disease [13], epilepsy [14], depression [15-16], addiction [17-19], and Alzheimer's Disease [20-21], the efficacy of DBS is being explored. The most pronounced beneficial effects have been observed with DBS of the subthalamic nucleus (STN) in PD patients.

Mechanism(s) behind the effects of deep brain stimulation is not fully unraveled:
The mechanisms by which DBS improves the symptoms, has been mostly investigated in PD individuals and animal models with emphasis on the "rate", and "synchronized oscillations" hypotheses [22-23]. In PD, a specific region in the basal ganglia, the STN, exhibits a continuous, abnormal, *burst firing rate* at the single-cell electrophysiological level [24-25] and *synchronized oscillations* with a frequency in the β-range (13–30 Hz) at the level of local field potentials [26], leading to characteristic changes in neuronal firing rates and patterns [27]. At stimulation settings commonly used in clinical practice, DBS decreases spontaneous firing of neuronal populations and drives axonal projections near the electrode [22-23]. These, modulate the pathological activity and replace it with a regular pattern of discharge with intervals of burst activity [28]. However, the exact mechanism(s) by which, DBS normalizes electrical activity in the basal ganglia and exerts beneficial effects on PD symptoms remain unknown. Thus far, research efforts have led to varying outcomes, which cannot be explained by the above-mentioned concept. For instance, based on the rate hypothesis, increased output from the Globus Pallidus internus (GPi) would cause PD symptoms, predicting that STN-DBS suppresses its output [29, 30]. However, STN-DBS is found to increase neuronal activity [31] and glutamate release in the GPi in patients [32]. Furthermore, the relationship between β-oscillations and PD symptoms has been shown to be more complex than initially thought [33]. Therefore, it appears that besides changes in firing rate and pattern of activity, other neuronal processes are involved. A potential mechanism would be a change in the neurochemical properties of the STN neurons, which are known to be exclusively glutamatergic, and its downstream regions. Neurotransmitter identity (type of a neurotransmitter that a neuron produces) of the neurons has been thought to be fixed throughout life, but environmental stimuli can drive behaviorally relevant transmitter switching in the mature brain thorough a recently discovered phenomenon, termed neurotransmitter respecification [2-5]. Our recent research indicates that DBS of the anterior nucleus of the thalamus enhances the number of dopaminergic neurons in the ventral tegmental area, providing evidence for neurotransmitter switching [1].

Neurotransmitter respecification in the mature brain:
Evidence for neurotransmitter respecification in the mature brain has been available for many years but has received surprisingly little attention [34-38]. For instance, early studies in primates demonstrated that the number of gamma-aminobutyric acid (GABA)-ergic neurons in the neocortex of primates is regulated by environmental stimuli [37-38]. Strong evidence for transmitter switching in the mature brain comes from a recent study examining the populations of interneurons in the adult rat hypothalamus, which switched between dopamine and somatostatin expression in response to exposure to short- and long-day photoperiods [3] Notably, in this study the changes in photoperiods are rather extreme than routine changes in day-night cycle that cause distress to the subjects.

Interestingly, in rodent and primate models of PD, lesioning dopaminergic neurons in the substantia nigra pars-compacta (SNc) can lead to the appearance of newly dopaminergic neurons, possibly via neurotransmitter switching from GABA to dopamine [39-40] in an activity-dependent manner [41]. These neurons have similar projection patterns to dopaminergic neurons in the SNc [42-44]. It will be of considerable interest to determine how widespread this process is in the PD brain and how PD symptoms are related to these changes. We hypothesize that STN-DBS triggers phenotype switching of STN neurons from glutamatergic to GABAergic phenotype and/or recruitment of newly dopaminergic neurons from the substantia nigra pars-reticulata (SNr) and/or change in the neuronal expression pattern of GABA/glutamate in the motor cortex and globus pallidus externus (GPe) neurons.

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?

Evidence for neurotransmitter switching has been accumulating steadily, both in the developing nervous system and in the adult brain, with observations of transmitter addition, loss, or replacement of one

transmitter with another. Natural stimuli can drive these changes in transmitter identity, with matching changes in postsynaptic transmitter receptors. Strikingly, they often convert the synapse from excitatory to inhibitory or vice versa, providing a basis for changes in behavior in those cases in which it has been examined. Thus, it has become clear that electrical activity is a factor that can induce transmitter switching, not only during development but in the mature nervous system as well.

We will test the hypothesis that behavioral changes following STN-DBS are mediated by neurotransmitter respecification in the STN and connected regions. These experiments will broaden our understanding of the mechanisms of DBS and will help to improve its current applications and develop new ones.
Our key objectives are to determine:

I. What is the extent of transmitter respecification before and after DBS in an animal model of PD?
II. Which circuits are involved and how? In particular we will address which of the followings will play a role; the local neurons at the target, downstream limbic or motor circuits or the brain region far away from the DBS target.
III. What is the impact of this transmitter respecification on behavioral parameters?

The electrophysiological, anatomical (objective I), behavioral and optogenetics (objectives III) experiments will be performed in our laboratories in Maastricht for approximately 2 years. The molecular biology and Ca2+ imaging experiments will be performed at UCSD (objective II). All techniques are established and running routinely in both laboratories. With regard to optogenetics setup in UM, the setup has been purchase, the safety permissions are obtained.

Our strong scientific back ground, well stablished infrastructures and good collaborators makes the above mentioned aims achievable.
This project is evaluated critically by NWO scientific board and awarded with VENI talent fellowship (2015).

3.3 Relevance

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

At present, thousands of patients with PD have been implanted with DBS electrodes and their number is expected to increase. The success of STN-DBS is based on solid scientific evidence derived from animal research in the 1980's and 1990's [50]. However, the exact mechanism behind the effects of DBS in PD is not well known. In this respect, I have introduced a potential mechanism, which can be used to understand the disease symptoms and in turn optimize and monitor the therapeutic effects.
In this application, we propose an in-depth investigation of the recently discovered mechanism of neuroplasticity, neurotransmitter respecification in PD. From a scientific and clinical point of view, there is a foreseeable possibility of identifying novel mechanisms of brain function that would facilitate the accurate targeting of the stimulation site in the brain and management of the psychiatric, cognitive and motor effects of DBS in patients with neurological disorders. Understanding the essence and extent of neurotransmitter respecification in local and remote neural elements following the application of electrical current will clarify the main components driving the therapeutic benefit, and the mechanisms that facilitate and that work at cross-purposes in patients. These will direct more rational and effective use of DBS and unleash its full therapeutic potential. Our translational DBS group (Prof. Temel) at MUMC will facilitate direct utilization of the scientific outcomes in clinical practice in a relatively short period of time (3-5 years). Neuronal mechanisms behind the therapeutic and side effects of DBS are not entirely known. In fact, many side effects cannot be explained by current knowledge. Investigating the neurotransmitter switching will reveal the changes in different areas and networks. For instance, if it turns out that STN DBS changes the monoaminergic system functionality, it explains why some PD patients suffer from mood/affect disturbances after DBS. This knowledge can be used to adjust the stimulation paradigms to avoid affecting monoaminergic system.
This group, comprised of integrated departments of neuroscience, neurosurgery and neurology, provides a unique team to conduct translational DBS studies. Close collaboration and communication between basic scientists and clinicians in our team has led to a successful implementation of preclinical findings in clinical DBS during the past years.

3.4 Research strategy

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

The project will consist of three independent experiments, related to every objective. Experiments I, II and III involve 3 inter-related sections, which together will provide a multi-level, interdisciplinary investigation of how STN-DBS affects the neurotransmitter identity of cells in the STN and related regions, which are linked to DBS-induced behavioral changes.
We will test the hypothesis that DBS modifies GABA, glutamate and monoamine-dependent behavioral outputs linked to mood, cognition and motor behavior; and that these effects involve the stimulation-derived neurotransmitter respecification in the STN, SNc, SNr, DRN and the neocortex in PD. The microcircuits that are linked to the behavioral effects of the STN-DBS will be selectively challenged (excitation/inhibition) by means of optogenetics to further verify the role of neurotransmitter respecification in mediating the behavioral effects of DBS.
Rats are preferred to mice in neuroscience research for many reasons, including the natural advantages of the rat model, the long history of pioneering development and validation of rat behavioral tasks and readout methods. However, due to following reasons we have to use mice for objectives I, II and III:

1- Genetically modified (Cell specific Cre recombinase) rats are not available and not established scientifically, whilst there are varieties of different mouse lines commercially available besides neurotoxic models.
2- Optogenetics experiments are more feasible in mice than rats.

Although, it should be noted that there is an ongoing rapid advance not only in optogenetics but also in rat genetic tools. This field of neuroscience is expected to continue to grow rapidly. Together with associated enabling technologies, rat optogenetics will likely play a crucial role in contributing to neuroscience research. However, application of optogenetics to the rat system is lagging behind applications to the mouse system by several years (56).

The electrophysiological, anatomical (objectives I), behavioral and optogenetics (objective III) experiments will be performed in our laboratories in Maastricht for approximately 2 years. The molecular biology and Ca^{2+} imaging experiments will be performed at UCSD (objective II), and will take approximately 6 months.

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.

Experiment I: We will identify the neurotransmitter respecification in neural circuit(s)/neuronal cell type(s) in PD and following STN-DBS, by means of combined immunohistochemical (IHC)/stereological and *in vivo* single unit recording methods. Experiments will focus on input and output structures of the STN, which are known to play a role in PD pathology.

The following sets of experiments will be conducted:

Four mice lines will be used; GABA-Cre, TH-Cre, 5-HT-Cre and Glu-Cre.
GABA- Cre mouse line expresses Cre specifically in GABA-ergic cell population.
TH-Cre mouse line expresses Cre specifically in Dopaminergic cell population.
5HT-Cre mouse line expresses Cre specifically in Serotonergic cell population.
Glu-Cre mouse line expresses Cre specifically in Glutamatergic cell population.

Mice will undergo stereotactic surgery and electrode implantation in the STN. Every mouse line will have a PD group which is induced by IP administration of MPTP neurotoxin. After recovery period, combined single cell recording and juxtacellular labeling will be performed under anesthesia to compare the electrophysiological and neurotransmitter finger-prints of the cells in the STN, SNc, SNr and the neocortex following neurotransmitter respecification. Afterwards, the subjects will be perfused transcarially.
We will conduct IHC on postmortem tissues to investigate neurotransmitter respecification in the STN, SNc, SNr, EP and the neocortex in PD and following STN-DBS.

The behavioral and molecular techniques selected for this study are among the ones that have contributed most to our current knowledge about the mechanism of action of DBS and its beneficial/side effects in Parkinson's disease (PD).

Experiment II: The set of experiments to achieve objective 2 will be conducted in UCSD, San Diego,

USA under local regulations.

Experiment III: The microcircuits that are linked to the behavioral effects of the STN-DBS will be selectively challenged (excitation/inhibition) by means of optogenetics to further verify the role of neurotransmitter respecification in mediating the behavioral effects of DBS.

Targeted expression of genes to specific cell types can be robustly achieved with the Cre–loxP system. Recent studies have used 'double-floxed' inverted open reading frame (DIO) viral vectors to achieve high specificity and expression levels [51].
The most commonly used strategy to date for the expression of ChR2 in brain tissue is through viral transduction. Viral vectors driving ChR2 expression can be delivered directly into specific brain regions with robust transduction efficacy and limited tissue damage. Adeno-associated viruses (AAVs) provide extensive spatial spread and high expression levels (52, 55).
The use of a viral DIO construct allows for expression of ChR2 exclusively in the subpopulation of neurons that expresses Cre recombinase. A number of transgenic, BAC and knock-in mouse lines, now commercially available, express Cre in specific populations of excitatory neurons (e.g., CW2, T29-1, T29-2, respectively) or inhibitory interneurons (e.g., PV-Cre). The specificity of ChR2 expression conferred by the DIO construct is very high (52-54). A fluorescent tag, such as mCherry fused to ChR2, allows post-hoc identification and mapping of ChR2-expressing cells for direct assessment of the specificity of expression.
In AAV DIO ChR2-mCherry, two incompatible loxP variants flank an inverted version of ChR2 fused to the fluorescent marker mCherry. In the presence of Cre, a stochastic recombination of either loxP variant takes place27, resulting in the inversion of ChR2-mCherry into the sense direction, followed by expression of the light-activated channels. Cre-dependent expression of light-activated channels or other genes is particularly well suited for targeting expression to cell types that lack identified promoter sequences (51).

The following sets of experiments will be conducted:

i) Mice will undergo stereotactic implantation of a cannula for virus injection and optogenetics probe insertion. Viral vectors will be utilized to induce expression of light-sensitive ion channels in the microcircuits that are identified to undergo neurotransmitter respecification in PD and following DBS. Targeted expression of genes to specific cell types can be robustly achieved with the Cre–loxP system. Recent studies have used 'double-floxed' inverted open reading frame (DIO) viral vectors to achieve high specificity and expression levels [51]. Thereafter these circuits will be challenged by means of optogenetics (instead of DBS) and mice (PD and healthy) and mice will be tested in behavioral settings.

ii) Behavioral experiments (to test PD related behavioral changes) will commence using the open-field test to evaluate locomotion and anxiety and a rotarod will be used to examine motor coordination. Moreover, we will use the elevated zero-maze to measure anxiety-related behavior and the Y-maze test to assess memory. At the end of the experiments, mice will be perfused transcardially.

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 project will consist of 3 inter-related sections, which together will provide a multi-level, interdisciplinary investigation of how STN-DBS affects the neurotransmitter identity of cells in the STN and related regions, which are linked to DBS-induced behavioral changes:

1. To identify the neural circuit(s) and neuronal cell type(s) that undergoes neurotransmitter respecification in a rodent model of PD after STN-DBS (objective.1).
2. To unravel molecular pathways that mediate DBS-derived neurotransmitter respecification (objective.2).
3. To test the hypothesis that behavioral changes following STN-DBS are mediated by neurotransmitter respecification in the STN and associated circuits (objective.3).

The electrophysiological and anatomical experiments will focus on the neurotransmitter identity of the STN, SNc, SNr, entopeduncular nucleus (rodent homologue of the primate GPi) and neocortical neurons

before and after STN-DBS in a rodent model of PD. We will apply STN-DBS using the stimulation parameters that have been shown to be effective in animal models and clinical settings. Control experiments will use electrodes implanted but not stimulated. All experiments will be conducted in three transgenic mouse lines specifically expressing green fluorescent protein (*GFP)* in glutamatergic, GABAergic or dopaminergic neurons. Thereafter these circuits will be challenged by means of optogenetics (instead of DBS) and mice (PD and healthy) will be tested in behavioral settings. Using these animal models will enable me to trace the neurotransmitter identity of the neurons before, during and after DBS. All experiments will be performed in stimulation on/off conditions in a neurotoxin (MPTP)-induced mouse model of PD and corresponding controls.
Based on our finding in experiment I, it might turn out that only one or some of the neurotransmitter systems (GABA, Glutamate, Dopamine and/or Serotonin) has undergone neurotransmitter switching. In this case we will conduct experiments II and III on one or some of the mouse lines. Experiment III is not depended on the out comes from experiment II, which is designed to investigate the mechanisms behind neurotransmitter switching. Experiment III will not be conducted if there is no positive outcome from experiment I in each mouse line. Experiment IV is an independent experiment.

Literature references

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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	Investigating the extent of transmitter respecification before and after DBS in an animal model of PD.
2	Studying the impact of this transmitter respecification on behavioral parameters in an animal model of PD.
3	
4	
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

3.

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	Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of deep brain stimulation in Parkinson's Disease
1.2	Looptijd van het project	5 jaar
1.3	Trefwoorden (maximaal 5)	Ziekte van Parkinson; diepe hersenstimulatie (deep brain stimulation, DBS); neurotransmitter verandering

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

X	Fundamenteel onderzoek
	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)

Diepe hersenstimulatie is een veel toegepaste therapie bij patiënten met neurologische en psychiatrische ziektebeelden, zoals de ziekte van Parkinson. Echter de effecten zijn niet optimaal en bijwerkingen kunnen optreden. Wij willen deze therapie verbeteren door onderzoek naar de recent ontdekte verandering in de identiteit van hersencellen a.g.v. elektrische stimulatie.

3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	Wetenschappelijke interesse: het definiëren van de hersencircuits die betrokken zijn bij de therapeutische effecten en bijwerkingen van diepe hersenstimulatie (deep brain stimulation, DBS). Zo krijgen we een fundamenteel begrip van hoe DBS de symptomen van de ziekte van Parkinson onderdrukt. Sociaal belang: Een beter begrip van de mechanismen van DBS zal de therapie verbeteren en bijwerkingen verminderen. Daarnaast kan deze nieuwe kennis de weg effenen voor de ontwikkeling van alternatieve en waarschijnlijk minder invasieve therapieën voor neurologische en/of psychiatrische stoornissen.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	Er zullen maximaal 800 volwassen muizen voor deze studie gebruikt worden.
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	Ongerief veroorzaakt door experimentele procedures, zoals chirurgie, meten van hersenactiviteit en gedragstesten. Ongerief veroorzaakt door het opwekken van het Parkinson (PD) model, zoals verminderde eetlust en motivatie.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	De mate van ongerief voor dit onderzoeksproject wordt geclassificeerd als matig. Post-operatieve pijn wordt bestreden met adequate pijnstilling. De stimulatie wordt dusdanig ingesteld dat de dieren er geen hinder van ondervinden.
3.6	Wat is de bestemming van de dieren na afloop?	Aan het eind van de experimenten zullen de dieren diepe anesthesie krijgen voor het meten van de hersenactiviteit, waarna de dieren op een humane manier worden geëuthanaseerd om het brein op cel niveau te onderzoeken.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	We zullen de hypothese testen dat gedragsveranderingen na DBS worden veroorzaakt door neurotransmitter verandering. Deze hypothese kan niet getoetst worden middels humaan onderzoek omdat: 1. Complexe gedragsanalyse van cognitie, geheugen, angst en stemming tijdens de verschillende manieren van diepe hersenstimulatie niet ethisch is bij de mens 2. Het brein niet tot op cel niveau te onderzoeken is bij de mens. 3. De markers die gebruikt worden om de neurotransmitter veranderingen aan te tonen niet veilig in humane studies te gebruiken zijn. Deze hypothese kan tevens niet getest worden wanneer wij computer modellen, celculturen gebruiken, of lagere diersoorten gebruiken, doordat wij de specifieke neurotransmitter productie binnen het hersennetwerk niet kunnen modelleren.
4.2	**Vermindering** Leg uit hoe kan worden	Wij zullen het aantal dieren in dit onderzoek beperken tot een minimum

	verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	door een statistische poweranalyse. Uit onze eigen ervaring en een literatuuroverzicht weten we dat er verlies kan ontstaan van dieren als gevolg van de ziekte van Parkinson en door de operaties. We hebben de poweranalyse hierop aangepast.
4.3	**Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Het gebruikte diermodel bootst de populatie van patiënten met de ziekte van Parkinson het beste na, is vaak gebruikt en biedt mogelijkheden voor een gedetailleerde analyse van gedrag tot op celniveau welke niet mogelijk is met andere modellen. Vergeleken met andere modellen, kunnen wij door middel van dit diermodel de ziekte van Parkinson relatief snel induceren, welke de tijd van de dieren in het experiment minimaliseert.
	Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	Alle experimentele procedures worden uitgevoerd door ervaren en geschoolde onderzoekers en verzorgers. Operaties worden uitgevoerd onder algehele anesthesie met adequate pijnbestrijding. Tijdens de postoperatieve herstelperiode worden de dieren zorgvuldig gecontroleerd. De dieren met ziekte van Parkinson worden nauwlettend gevolgd en verzorgd om ernstig ongemak te voorkomen. Tevens zullen we minimaal invasieve benaderingen gebruiken m.b.t de operaties. Ook zullen we de huisvesting en de mate van zorg aanpassen aan de behoeftes van de dieren in de verschillende stadia van de experimenten.

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'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht University

1.3 List the serial number and type of animal procedure.

Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.

Serial number	Type of animal procedure
1	Investigating the extent of transmitter respecification before and after DBS in an animal model of PD.

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 **experimental approach** of this study is to apply Deep Brain Stimulation (DBS) in subthalamic nucleus (STN) of parkinsonian (PD) and sham control mice. Consequently, we will identify the neurotransmitter respecification in neural circuit(s)/neuronal cell type(s) in PD and following STN-DBS, by means of combined immunohistochemical (IHC)/stereological and in vivo single unit recording methods. Experiments will focus on input and output structures of the STN, which are known to play a role in PD pathology.

The electrophysiological and anatomical experiments will focus on the neurotransmitter identity of the STN, SNc, SNr, entopeduncular nucleus (rodent homologue of the primate GPi) and neocortical neurons before and after STN-DBS in a rodent model of PD. We will apply STN-DBS using the stimulation parameters that have been shown to be effective in animal models and clinical settings. Control experiments will use electrodes implanted but not stimulated. All experiments will be conducted in four transgenic mouse lines containing Cre recombinase in specific cell types. Using viral vectors, mice will express fluorescent protein (*GFP or EYFP)* in glutamatergic, GABAergic or monoaminergic neurons. Using these animal models will enable us to trace the neurotransmitter identity (type of neurotransmitter that a given cell produces e.g., serotonergic, dopaminergic etc.,) of the neurons before, during and after DBS. All experiments will be performed in stimulation on/off conditions in a neurotoxin (MPTP)-induced mouse model of PD and corresponding controls (figure 1).

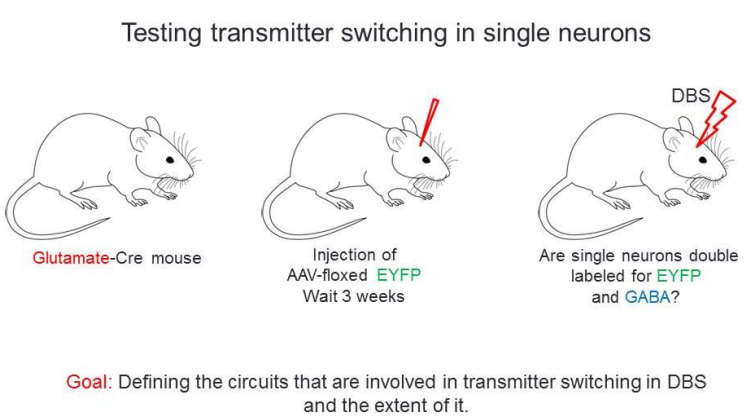

Figure 1: Schematic representation of experiment I. Similar experiments will be conducted in TH, GABA and 5HT-Cre mice lines and corresponding controls in PD and healthy conditions.

The primary outcomes: we will use IHC to investigate neurotransmitter respecification in the STN, SNc, SNr, EP and the neocortex in PD and following STN-DBS. According to the disease pathology and the therapeutic effects of DBS, we expect the following outcomes:
a) alterations in GABA expression in the basal ganglia and related behavioral changes.
b) alterations in dopamine expression in the SNc and SNr and related behavioral changes.
c) alterations in the expression pattern of glutamate/GABA-ergic neurons in the neocortex and related behavioral changes.
d) alterations in GABA expression in the EP and SNr and related behavioral changes.
Double labeling these cell populations with GFP will allow us to show that the neurons have undergone neurotransmitter respecification.

i) Behavioral experiments will commence using different test batteries to evaluate the therapeutic and side effects of DBS in the STN, related to neurotransmitter switching.

ii) The changes in the numbers of neurons expressing different neurotransmitters will be quantified by means of high precision design-based stereology.

iii) Combined single cell recording and juxtacellular labeling will be performed to compare the electrophysiological and neurotransmitter finger-prints of the cells in the STN, SNc, SNr and the neocortex following neurotransmitter respecification.

Table 1. Summary of the different behavioral tests and readout parameters.

Test paradigm	Item	Readout parameter	Discomfort
Object location task	memory	ratio of time spent at object in new versus old location.	Mild discomfort, results in increased anxiety
Skinner box paradigms e.g. reaction time and attention tasks	cognition	reaction time, incorrect responses, correct responses, premature responses	Moderate discomfort, animals will receive restricted feeding regimes or deprivation
Rotarod	locomotion	time spent on rotating cylinder	Mild discomfort, results in increased anxiety
Sucrose	mood	volume of sucrose intake corrected	Mild discomfort,

intake/preference		for animal weight.	results in increased anxiety
Open field	anxiety and locomotion	total distance travelled, average speed and time spent in corners.	Mild discomfort, results in increased anxiety
Elevated zero maze	anxiety	time spent in closed arms	Mild discomfort, results in increased anxiety

Though all animals will undergo a battery of behavioral testing, related to PD symptoms. These tests will be used to assess mood, cognitive and motor functions. The selection of tests may change in the course of the study due to analyses of previous tests. We therefore study STN DBS in different Cre mouse lines to find the behavioral impact of neurotransmitter switching on the behavior. In case of observing discomfort in the animals with one or some the behavioral tasks, an alternative test will be used.
If multiple behavioral tests are suitable and both could answer the research question of interest, we will select the test with the least degree of discomfort for following studies. Cumulative discomfort caused by consequent behavioral testing will be minimized by a wash out period appropriately for the different tests. Each subject will undergo maximum of 6 behavioral tests related to locomotion, mood, anxiety, cognition and memory. The open field test for locomotion and anxiety; the Rotarod test for locomotion; the elevated zero maze for anxiety; the sucrose intake test for mood; object location test for memory and the Skinner box test for cognition. If the open field test reveals the changes in anxiety and locomotion, the elevated zero maze and the Rotarod tests will not be conducted.

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

This study is a chronic DBS experiment and involves DBS, video and electrophysiological recordings, and behavioral tests in freely moving and anesthetized mice. For a summary of the experimental groups see section 2B. The procedures proposed in this study are comparable to those published in literature and previous (un)published studies in which we used similar DBS and behavioral testing paradigms in animal models for neurodegenerative diseases. Based on our previous experience, we estimate a maximal experimental time of 6 months per animal [1-4].

MPTP mouse model of Parkinson's disease

Mice in PD group will be treated with MPTP one week prior to the surgery [5, 6].

Surgery and behavioral experiments
AVV-Floxed EYFP will be infused by means of stereotactic surgery bilaterally in the:
STN in Glu-Cre mice, SNC and SNr in TH-Cre mice, STN and cortex in GABA-Cre mice and the DRN in 5HT-Cre mice. Each mice line will have PD and sham control. Thereafter, mice will undergo stereotactic implantation of DBS electrodes in the STN. All groups will receive 2-4 weeks recovery to allow the Cre recombinase to unpack the EYFP or GFP in order to express the florescent protein in specific cell groups.
Surgical procedures will be conducted under general anesthesia. Sham animal will undergo the same surgical procedure, but are not stimulated through the electrodes.

Subsequently, animals will be subjected to behavioral tests for motor, memory, cognition, mood and anxiety. As mentioned in section 2A, the choice of behavioral test may vary in course of the study. Behavioral tests will be performed in both DBS *on* and *off* conditions (for maximally 6 months of behavioral testing). Experimental groups consist of animals that receive high (130 Hz) frequency DBS in stimulation paradigms, which are used in our previous PD research [2, 4].

Single cell electrophysiology and juxtacellular recording
At the end of the behavioral experiments mice will be subjected to single cell electrophysiology under anesthesia (terminal experiment).

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

To minimize the number of animals, we will consider and apply published studies, previous studies by our group and a power analyses. Moreover, this study is designed in several levels and phases. Based on this phase-designed approach we will test our hypothesis step by step in smaller badges of animals but not at once. This approach will prevent unnecessary/extra experiments.

B. The animals

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

Animals

These experiments require cell specific Cre containing adult mice, as these animals are used routinely in previous optogenetic studies [7].

Gender

We will only use male mice, since previous studies have shown that the oestrogen cycle can interfere with brain neurochemistry. Sex hormones have been implicated in neurite outgrowth, synaptogenesis, dendritic branching, myelination and other important mechanisms of neural plasticity. Recent evidences from animal experiments and human studies report interactions between sex hormones and the dominant neurotransmitters, such as serotonin, dopamine, GABA and glutamate. Accumulating data during physiological and pathological conditions and discuss currently conceptualized theories on how sex hormones potentially trigger neuroplasticity changes through these four neurochemical systems. Many brain regions have been demonstrated to express high densities for estrogen- and progesterone receptors, such as the amygdala, the hypothalamus, and the hippocampus. These changes have been linked to differences in behavior, neurochemical patterns and hippocampal structure to a changing hormonal environment. Physiologically occurring hormonal transition periods changes in sex hormones influence functional connectivity, neurotransmission and brain structure in vivo [8-12].

In this study we will use 10-12 weeks old mice. Based on our experience, mice at this age are more suitable for electrode/cannula implantation. In older mice (or rats) the sutures position change on the skull. This makes it difficult to navigate stereotactic implantation. Besides, jaw muscles grow towards midline by aging and therefore leave a little space for electrode/cannula construct.

Number of animals

We estimation on the total number of 25 animals/group is based on our previous experience with behavioral studies and a literature review [6].

We estimate a maximum number of 400 animals for this study.

Table 1: description of the experimental groups.

Experimental groups for Glu-Cre line	*Number of mice/group*
Naïve Sham	25
MPTP Sham	25
Naïve electrical DBS	25
MPTP electrical DBS	25
Experimental groups for GABA-Cre line	
Naïve Sham	25
MPTP Sham	25
Naïve electrical DBS	25
MPTP electrical DBS	25
Experimental groups for TH-Cre line	
Naïve Sham	25
MPTP Sham	25
Naïve electrical DBS	28
MPTP electrical DBS	25
Experimental groups for 5HT-Cre line	
Naïve Sham	25

MPTP Sham	25
Naïve electrical DBS	25
MPTP electrical DBS	25
Total	400

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 primary aim of this study is to test the hypothesis that behavioral changes following STN-DBS are mediated by neurotransmitter respecification in the STN and connected regions.

These aims cannot be achieved by use of in vitro experiments or computer modelling, because these models do not allow for analysis of behavior and do not represent the organization of a complex neuronal network in a complex biological system such as the brain. In previous studies [13, 14] we have successfully shown that by using DBS in several rat models of neurodegenerative diseases, we can model the therapeutic and behavioral side effects of DBS. The current study can also not be performed in humans because of the following: 1. Complex behavioral analysis of cognition, memory, anxiety and mood during different stimulation paradigms compared to sham operation is not ethical in humans 2. This project involve using Cre construct in specific cell types is not possible in human subjects. 3. The approach in this study requires using AVV viral vectors, which again restricted in human studies.

Reduction

We will limit the number of animals in this study to a minimum by using a power analysis. The power analyses are based on our primary outcome measure: behavioral improvement. By decreasing group-size we will under power this study which may lead to falls results. Besides, we expect variable responses to DBS as well as the neurotoxin that will be applied to induce PD in mice. Current group size will allow us to subdivide the subject based on their response and understand the neurobiological bases of their responses. In addition, applying a "phased design" is taken into account to reduce the number of animals used throughout the experiments. Specifically; if turns out that only one or some of the neurotransmitter systems (GABA, Glutamate, Dopamine and/or Serotonin) has undergone neurotransmitter switching in experiment "I", we will conduct experiments II and III on one or some of the mouse lines. We will take the following measurements to reduce loss of animals: Most drop out is expected for mice with PD in compare to the healthy subjects. For this reason, we will conduct a daily welfare evaluation and tracking with special attention on PD mice; in particular their nutrition and body weight.

Refinement

We choose the MPTP animal model for PD, since animals by this model exert behavioral phenotype of the PD, therefore are likely to show PD-related neurotransmitter switching. Based on our literature review, MPTP is the best method to induce PD in mice [15-17]. As a matter of fact, choosing the animal model, with resembles the human condition most, results in shorter and less complex behavioral experiments. Moreover, MPTP can be administered intraperitoneally. This will thus decrease the time that the animals

are in experiment, unlike surgical administration, which adds few weeks to the experiment. Additionally, we have put extra efforts to design the experiment as such that cause less distress to the animals. In particular, the best and most up to date surgical procedures, electrode construct, drug administration methods and behavioral tests are planned. Moreover, we know from our own previous published/unpublished studies that which readout measures are more representative and useful. For instance; following observation of certain behavioral phenotype (e.g. impulsivity, depressive like behavior etc.,), what sort of analysis should be conducted. Knowing these, will shorten the duration of the experiments. The proposed experiment is dealing with both healthy and PD animals. An increased discomfort is expected for mice with PD symptoms in compare to the healthy subjects. For this reason, we will conduct a daily welfare evaluation and tracking with special attention on PD mice; in particular their nutrition and body weight. Finally, we will adapt the accommodation and the care to the need of the animals at the different time stages during the experiment.

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

Animals will be under general anesthesia during surgical procedures and will receive appropriate analgesics during the post-operative recovery period. The electrode implantation is similar to the implantation of stimulation electrodes in the clinic, performed under local anesthesia. These patients experience minor pain; therefore there is no need for analgesics. In laboratory animals, this is done under general anesthesia. Animals will receive Pre and post-operative analgesia. In addition, preoperative, local anesthetic will be injected at the site of incision. In experiment, if a mouse shows sign of pain, distress, infection or inflammation, it will be treated with analgesics, antibiotics or anti-inflammatory medications. We will prevent pain and discomfort by monitoring the animals during behavioral experiments. The injections and behavioral test will be conducted according to standard guidelines. Similarly, if animals show pain during any of the experimental procedures described, they will receive analgesics. The persons involved in this experiment possess a strong background of laboratory animal welfare experiences to minimize animal suffering, pain or fear.

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 concept of neurotransmitter switching in adult mammals is very new. Besides us, there is only one group (UCSD, San Diego, USA), that conducts research on this subject, whom we will collaborate with in this project. When combining neurotransmitter switching concept and DBS, there will be no possibility for duplication, meaning that we are aware of this filed and will not do an experiment, which has been done/being done by others. Notably, the novelty and originality of this project has been evaluated recently by the scientific board of NWO and granted with VENI research fellowship.

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.

All animals will be housed socially, unless fighting or damaging the electrode construct. The cages and water will be renewed once a week; and the weight of the animals will be measured and written down in a laboratory book.

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.

General anesthesia will be used for surgical procedures. Animals will be monitored daily during the experimental procedures stated in section 2A and will be checked for signs of pain or discomfort. Standard analgesic will be applied to relief suffering e.g. during the post-surgical recovery period.

I. Other aspects compromising the welfare of the animals

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

A possible adverse effect which might occur is loss of an electrode construct. Mice with PD symptoms might face adverse effects in particular their nutrition and body weight.
Viral tools used in this study are not infectious [7, 18]. The viral tools are applied locally in a very small quantity. Thus, do not induce any immune reaction.

Explain why these effects may emerge.

The electrode construct is fixed on the skull of the animal using dental cement. In the postoperative period the head skin of the animal will heal and grow around the electrode construct. However infection may still occur. Special care will be taken for mice with PD. However, due to lack of dopaminergic neurotransmission, some animals might have less motivation for food and thus lose weight.

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

Animals will be visually inspected daily during experiments. During recovery period after surgery, mice will be inspected several times a day and body weight will be measured every day. Recovery boost gel will be administered if animals are not gaining weight. Prophylactic antibiotic will be administered. In case of adverse effects, the experiment will be halted and the animals will be treated accordingly.

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.

In case of electrode detachment, severe illness or tumor growth human end point will be applied. More complications are expected for mice with PD symptoms in compare to the healthy subjects. Lesioning dopaminergic system will affect their nutrition and body weight. Neurotoxin induced PD model in this study is not considered as a progressive model. Majority of PD symptoms start to disappear slowly after several months. This is due to regeneration and sprouting of dopaminergic neurons. These experiments will be conducted before regeneration starts. Greater side effects are expected to appear during the first couple of weeks after neurotoxin infusion.

Lesioning the DA cells affects the animals' ability to ambulate and perform normal body functions, and these potential effects on health and well-being mandate additional steps to ensure humane animal care and use: if clinical signs of PD disables the animal to keep normal nutrition requirements despite special nursing and care, which are indicative for moderate exceeding discomfort, humane endpoint will be applied. Scoring on general impression (awareness, gait, performing species specific behavior, body condition and body composition, posture, fur/skin appearance, fascial expression), clinical signs of PD, body weight, hydration state and performance in behavior tasks will be used to define the humane endpoint. If those symptoms persists longer than 36 hours (despite treatment), mice will be euthanized. An animal welfare score will be used to evaluate the animal wellbeing.

Indicate the likely incidence.

We estimate the likely incidence at maximally 10%. However, the pilot and proof of principle studies described in section 2A are carried out to reduce this incidence in both this study and for the studies described in appendix 2 and 3.

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 expected level of discomfort is mild for all animals due to stereotactic surgery for implantation of the electrode construct, virus injection and behavioral testing. We consider the discomfort caused by the PD model to be moderate. Cumulative discomfort caused by these experiments is expected to be 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.

At the end of the experiments, all animals will be sacrificed to isolate the brain and perform histologic analysis. Histologic analysis of the brain is needed to check for the correct electrode localization and to study the neuroanatomical effects of DBS and correlate this to behavioral outcome.

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

References

1. Tan, S., et al., *Experimental deep brain stimulation in animal models.* Neurosurgery, 2010. **67**(4): p. 1073-9; discussion1080.
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5. Jackson-Lewis, V. and S. Przedborski, *Protocol for the MPTP mouse model of Parkinson's disease.* Nat Protoc, 2007. **2**(1): p. 141-51.

6. Shaw, V.E., et al., *Patterns of Cell Activity in the Subthalamic Region Associated with the Neuroprotective Action of Near-Infrared Light Treatment in MPTP-Treated Mice.* Parkinsons Dis, 2012. **2012**: p. 296875.
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9. Bredemann, T.M. and L.L. McMahon, *17beta Estradiol increases resilience and improves hippocampal synaptic function in helpless ovariectomized rats.* Psychoneuroendocrinology, 2014. **42**: p. 77-88.
10. Toffoletto, S., et al., *Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: a systematic review.* Psychoneuroendocrinology, 2014. **50**: p. 28-52.
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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'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Studying the impact of this transmitter respecification on behavioral parameters in an animal model of PD.

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 **experimental approach** of this study is to apply optogenetic DBS in the networks that involve in stimulation-derived neurotransmitter respecification in parkinsonian (PD) and sham control mice. Consequently, we will test the hypothesis that DBS modifies GABA, glutamate and monoamine-dependent behavioral outputs linked to mood, cognition and motor behavior; and that these effects involve the stimulation-derived neurotransmitter respecification in the STN, SNc, SNr and the neocortex in PD. The microcircuits that are linked to the behavioral effects of the STN-DBS (which will be defined in experiment I) will be selectively challenged (excitation/inhibition) by means of optogenetics to further verify the role of neurotransmitter respecification in mediating the behavioral effects of DBS. Targeted expression of genes to specific cell types can be robustly achieved with the Cre–loxP system. Recent studies have used 'double-floxed' inverted open reading frame (DIO) viral vectors to achieve high specificity and expression levels. Therefore, specific cell types will be modulated by means of optogenetic neuromodulation.

We will apply optogenetic neuromodulation using the stimulation parameters that have been shown to be effective in animal models [1, 2]. Optogenetic stimulation of the STN in rodent models of PD has shown to be effective in modulating cell firing pattern, local field potentials and oscillatory pattern of activity [1, 2]. Control experiments will use cannula implanted but not stimulated. The experiments will be conducted in four transgenic mouse lines with containing Cre recombinase construct in specific cell types. Using viral vectors, mice will express fluorescent protein (*GFP or EYFP) and channelrhodopsin* in glutamatergic, GABAergic or monoaminergic neurons. All experiments will be performed in stimulation

on/off conditions in a neurotoxin (MPTP)-induced mouse model of PD and corresponding controls (figure 1).

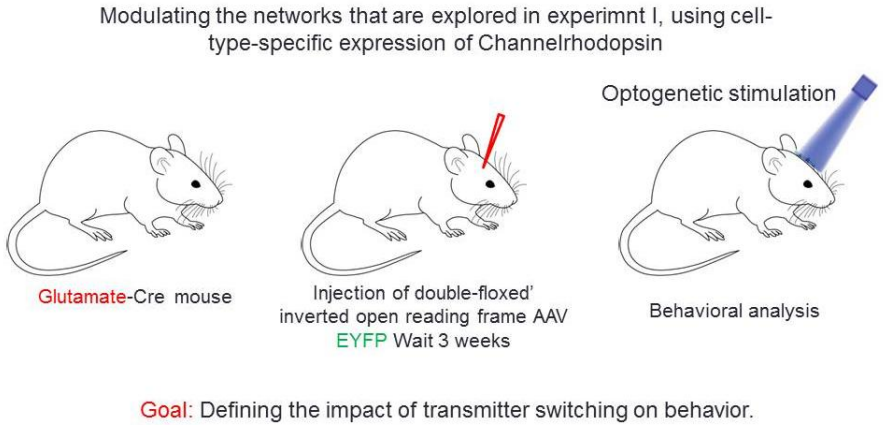

Figure 1: Schematic representation of experiment II. same experiment will be conducted in TH, GABA and 5HT-Cre mice lines and corresponding controls in PD and healthy mice.

The primary outcomes: we will use IHC to verify neurotransmitter switching in the STN, SNc, SNr, EP and the neocortex in PD and following optogenetic neuromodulation. According to the disease pathology and the therapeutic effects of DBS, we expect more beneficial outcomes (eg., improved dyskinesia) and less side effect (eg., depressive-like behavior) with optogenetic neuromodulation of specific cell types. The therapeutic and side effects will be evaluated using behavioral test battery.

i) Behavioral experiments will commence using different test batteries to evaluate the therapeutic and side effects of DBS, related to neurotransmitter switching.

ii) The changes in the numbers of neurons expressing different neurotransmitters will be quantified by means of high precision design-based stereology.

iii) Combined single cell recording and juxtacellular labeling will be performed to compare the electrophysiological and neurotransmitter finger-prints of the cells in the STN, SNc, SNr and the neocortex following neurotransmitter respecification.

Table 1. Summary of the different behavioral tests and readout parameters.

Test paradigm	Item	Readout parameter	Discomfort
Object location task	memory	ratio of time spent at object in new versus old location.	Mild discomfort, results in increased anxiety
Skinner box paradigms e.g. reaction time and attention tasks	cognition	reaction time, incorrect responses, correct responses, premature responses	Moderate discomfort, animals will receive restricted feeding regimes or deprivation
Rotarod	locomotion	time spent on rotating cylinder	Mild discomfort, results in increased anxiety
Sucrose intake/preference	mood	volume of sucrose intake corrected for animal weight.	Mild discomfort, results in increased anxiety
Open field	anxiety	total distance travelled, average	Mild discomfort,

	and locomotion	speed and time spent in corners.	results in increased anxiety
Elevated zero maze	anxiety	time spent in closed arms	Mild discomfort, results in increased anxiety

Though all animals will undergo a battery of behavioral testing, related to PD symptoms. These tests will be used to assess mood, cognitive and motor functions. The selection of tests may change in the course of the study due to analyses of previous tests. We therefore study optogenetics DBS in different Cre mouse lines to find the behavioral impact of neurotransmitter switching on the behavior. In case of observing discomfort in the animals with one or some the behavioral tasks, an alternative test will be used. If multiple behavioral tests are suitable and both could answer the research question of interest, we will select the test with the least degree of discomfort for following studies. Cumulative discomfort caused by consequent behavioral testing will be minimized by a wash out period appropriately for the different tests. Each subject will undergo maximum of 6 behavioral tests related to locomotion, mood, anxiety, cognition and memory. The open field test for locomotion and anxiety; the Rotarod test for locomotion; the elevated zero maze for anxiety; the sucrose intake test for mood; object location test for memory and the Skinner box test for cognition. If the open field test reveals the changes in anxiety and locomotion, the elevated zero maze and the Rotarod tests will not be conducted.

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

This study is a chronic DBS experiment and involves optogenetic DBS, video and electrophysiological recordings and behavioral tests in freely moving and anesthetized mice. For a summary of the experimental groups see section 2B. The procedures proposed in this study are comparable to those published in literature and previous (un)published studies in which we used similar DBS and behavioral testing paradigms in animal models for neurodegenerative diseases. Based on our previous experience, we estimate a maximal experimental time of 6 months per animal [3-6].

MPTP mouse model of Parkinson's disease

Mice in PD group will be treated with MPTP one week prior to the surgery [7, 8].

Surgery and behavioral experiments
Double-floxed inverted open reading frame viral vector will be infused to achieve targeted expression of genes to specific cell types by means of stereotactic surgery bilaterally in the:
STN in Glu-Cre mice, SNC and SNr in TH-Cre mice, STN and cortex in GABA-Cre mice and the DRN in 5HT-Cre mice. Each mice line will have PD and sham control. Thereafter, mice will undergo stereotactic implantation of guide cannula (to insert optogenetic probe). All groups will receive 2 - 4 weeks recovery to allow the Cre recombinase to unpack the EYFP or GFP in order to express the florescent protein in specific cell groups.
Surgical procedures will be conducted under general anesthesia. Sham animal will undergo the same surgical procedure, but are not stimulated through the cannulas.

Subsequently, animals will be subjected to behavioral tests for motor, memory, cognition, mood and anxiety. As mentioned in section 2A, the choice of behavioral test may vary in course of the study. Behavioral tests will be performed in both DBS *on* and *off* conditions (for maximally 6 months of behavioral testing). Experimental groups consist of animals that receive optogenetics DBS in stimulation paradigms, which are used in previous studies [3-6].

Single cell electrophysiology and juxtacellular recording
At the end of the behavioral experiments mice will be subjected to single cell electrophysiology under anesthesia (terminal experiment) (see SOP nr 5). Subsequently, animals are euthanized by either perfusion or decapitation as required for follow-up histological analysis of the brain.

Describe which statistical methods have been used and which other considerations have been taken into

account to minimize the number of animals.

To minimize the number of animals, we will consider and apply published studies, previous studies by our group and a power analyses. Moreover, this study is designed in several levels and phases. Based on this phase-designed approach we will test our hypothesis step by step in smaller badges of animals but not at once. This approach will prevent unnecessary/extra experiments.

B. The animals

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

Animals
We estimation on the total number of 25 animals/group is based on our previous experience with behavioral studies and a literature review [8].

Gender
We will only use male mice, since previous studies have shown that the oestrogen cycle can interfere with brain neurochemistry. Sex hormones have been implicated in neurite outgrowth, synaptogenesis, dendritic branching, myelination and other important mechanisms of neural plasticity. Recent evidences from animal experiments and human studies report interactions between sex hormones and the dominant neurotransmitters, such as serotonin, dopamine, GABA and glutamate. Accumulating data during physiological and pathological conditions and discuss currently conceptualized theories on how sex hormones potentially trigger neuroplasticity changes through these four neurochemical systems. Many brain regions have been demonstrated to express high densities for estrogen- and progesterone receptors, such as the amygdala, the hypothalamus, and the hippocampus. These changes have been linked to differences in behavior, neurochemical patterns and hippocampal structure to a changing hormonal environment. Physiologically occurring hormonal transition periods changes in sex hormones influence functional connectivity, neurotransmission and brain structure in vivo [9-13].
In this study we will use 10-12 weeks old mice. Based on our experience, mice at this age are more suitable for electrode/cannula implantation. In older mice (or rats) the sutures position change on the skull. This makes it difficult to navigate stereotactic implantation. Besides, jaw muscles grow towards midline by aging and therefore leave a little space for electrode/cannula construct.

Number of animals
The estimation on the total number of animals is based on our previous experience with DBS studies and a literature review. We expect a group size of animals 25 mice [8].
We estimate a maximum number of 400 animals for this study.
Table 1: description of the experimental groups.

Experimental groups for Glu-Cre line	*Number of mice/group*
Naïve Sham	25
MPTP Sham	25
Naïve optogenetic neuromodulation	25
MPTP optogenetic neuromodulation	25
Experimental groups for GABA-Cre line	
Naïve Sham	25
MPTP Sham	25
Naïve optogenetic neuromodulation	25
MPTP optogenetic neuromodulation	25
Experimental groups for TH-Cre line	
Naïve Sham	25
MPTP Sham	25
Naïve optogenetic neuromodulation	25
MPTP optogenetic neuromodulation	25
Experimental groups for 5HT-Cre line	
Naïve Sham	25
MPTP Sham	25

Naïve optogenetic neuromodulation	25
MPTP optogenetic neuromodulation	25
Total	400

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 primary aim of this study is to test the hypothesis that behavioral changes following STN-DBS are mediated by neurotransmitter respecification in the STN and connected regions.

These aims cannot be achieved by use of in vitro experiments or computer modelling, because these models do not allow for analysis of behavior and do not represent the organization of a complex neuronal network in a complex biological system such as the brain. In previous studies [14, 15] we have successfully shown that by using DBS in several rat models of neurodegenerative diseases, we can model the therapeutic and behavioral side effects of DBS. The current study can also not be performed in humans because of the following: 1. Complex behavioral analysis of cognition, memory, anxiety and mood during different stimulation paradigms compared to sham operation is not ethical in humans 2. This project involve using Cre construct in specific cell types is not possible in human subjects. 3. The approach in this study requires using AVV viral vectors, which again restricted in human studies.

Reduction

We will limit the number of animals in this study to a minimum by using a power analysis. The power analyses are based on our primary outcome measure: behavioral improvement. By decreasing group-size we will under power this study which may lead to falls results. Besides, we expect variable responses to DBS as well as the neurotoxin that will be applied to induce PD in mice. Current group size will allow us to subdivide the subject based on their response and understand the neurobiological bases of their responses. In addition, applying a "phased design" is taken into account to reduce the number of animals used throughout the experiments. Specifically; if turns out that only one or some of the neurotransmitter systems (GABA, Glutamate, Dopamine and/or Serotonin) has undergone neurotransmitter switching in experiment "I", we will conduct experiments II and III on one or some of the mouse lines. We will take the following measurements to reduce loss of animals: Most drop out is expected for mice with PD in compare to the healthy subjects. For this reason, we will conduct a daily welfare evaluation and tracking with special attention on PD mice; in particular their nutrition and body weight.

Refinement

We choose the MPTP animal model for PD, since animals by this model exert behavioral phenotype of the PD, therefore are likely to show PD-related neurotransmitter switching. Based on our literature review, MPTP is the best method to induce PD in mice [16-18]. As a matter of fact, choosing the animal model, with resembles the human condition most, results in shorter and less complex behavioral experiments. Moreover, MPTP can be administered intraperitoneally. This will thus decrease the time that the animals are in experiment, unlike surgical administration, which adds few weeks to the experiment. Additionally, we have put extra efforts to design the experiment as such that cause less distress to the animals. In particular, the best and most up to date surgical procedures, cannula construct, drug administration

methods and behavioral tests are planned. Moreover, we know from our own previous unpublished study that which readout measures are more representative and useful. For instance; following observation of certain behavioral phenotype (e.g. impulsivity, depressive like behavior etc.,), what sort of analysis should be conducted. Knowing these, will shorten the duration of the experiments. The proposed experiment is dealing with both healthy and PD animals. An increased discomfort is expected for mice with PD symptoms in compare to the healthy subjects. For this reason, we will conduct a daily welfare evaluation and tracking with special attention on PD mice; in particular their nutrition and body weight. Finally, we will adapt the accommodation and the care to the need of the animals at the different time stages during the experiment.

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

Animals will be under general anesthesia during surgical procedures and will receive appropriate analgesics during the post-operative recovery period. The cannula implantation is similar to the implantation of cannula in the clinic, performed under local anesthesia. These patients experience minor pain; therefore there is no need for analgesics. In laboratory animals, this is done under general anesthesia. Animals will receive Pre and post-operative analgesia. In addition, preoperative, local anesthetic will be injected at the site of incision. In experiment, if a mouse shows sign of pain, distress, infection or inflammation, it will be treated with analgesics, antibiotics or anti-inflammatory medications. We will prevent pain and discomfort by monitoring the animals during behavioral experiments. The injections and behavioral test will be conducted according to standard guidelines. Similarly, if animals show pain during any of the experimental procedures described, they will receive analgesics. The persons involved in this experiment possess a strong background of laboratory animal welfare experiences to minimize animal suffering, pain or fear.

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 concept of neurotransmitter switching in adult mammals is very new. Besides us, there is only one group (UCSD, San Diego, USA), that conducts research on this subject, whom we will collaborate with in this project. When combining neurotransmitter switching concept and DBS, there will be no possibility for duplication, meaning that we are aware of this filed and will not do an experiment, which has been done/being done by others. Notably, the novelty and originality of this project has been evaluated recently by the scientific board of NWO and granted with VENI research fellowship.

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.

All animals will be housed socially, unless fighting or damaging the cannula construct.The cages and water will be renewed once a week; and the weight of the animals will be measured and written down in a laboratory book.

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.

General anesthesia will be used for surgical procedures. Animals will be monitored daily during the experimental procedures stated in section 2A and will be checked for signs of pain or discomfort. Standard analgesic will be applied to relief suffering e.g. during the post-surgical recovery period.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?
A possible adverse effect which might occur is loss of an implanted construct. Mice with PD symptoms might face adverse effects in particular their nutrition and body weight.
Viral tools used in this study are not infectious [1, 2]. The viral tools are applied locally in very small quantity. Thus, do not induce any immune reaction.

Explain why these effects may emerge.

The cannula construct is fixed on the skull of the animal using dental cement. In the postoperative period the head skin of the animal will heal and grow around the construct. However infection may still occur. Special care will be taken for mice with PD. However, due to lack of dopaminergic neurotransmission, some animals might have less motivation for food and thus lose weight.

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

Animals will be visually inspected daily during experiments. During recovery period after surgery, mice will be inspected several times a day and body wait will be measured every day. Recovery boost gel will be administered if animals are not gaining weight. Prophylactic antibiotic will be administered. In case of adverse effects, the experiment will be halted and the animals will be treated accordingly.

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.

In case of cannula detachment, severe illness or tumor growth, humane end point will be applied. More complications are expected for mice with PD symptoms in compare to the healthy subjects. Lesioning dopaminergic system will affect their nutrition and body weight. Neurotoxin induced PD model in this study is not considered as a progressive model. Majority of PD symptoms start to disappear slowly after several months. This is due to regeneration and sprouting of dopaminergic neurons. These experiments will be conducted before regeneration starts. Greater side effects are expected to appear during the first couple of weeks after neurotoxin infusion.
Lesioning the DA cells affects the animals' ability to ambulate and perform normal body functions, and these potential effects on health and well-being mandate additional steps to ensure humane animal care and use: if clinical signs of PD disables the animal to keep normal nutrition requirements despite special

nursing and care, which are indicative for moderate exceeding discomfort, humane endpoint will be applied. Scoring on general impression (awareness, gait, performing species specific behavior, body condition and body composition, posture, fur/skin appearance, fascial expression), clinical signs of PD, body weight, hydration state and performance in behavior tasks will be used to define the humane endpoint. If those symptoms persists longer than 36 hours (despite treatment), mice will be euthanized. An animal welfare score will be used to evaluate the animal wellbeing.

Indicate the likely incidence.

We estimate the likely incidence at maximally 10%. However, the pilot and proof of principle studies described in section 2A are carried out to reduce this incidence in both this study and for the studies described in appendix 2 and 3.

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 expected level of discomfort is mild for all animals due to stereotactic surgery for implantation of the cannula, virus injection and behavioral testing. We consider the discomfort caused by the PD model to be moderate. Cumulative discomfort caused by these experiments is expected to be 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.

At the end of the experiments, all animals will be sacrificed to isolate the brain and perform histologic analysis. Histologic analysis of the brain is needed to check for the correct cannula localization and to study the neuroanatomical effects of DBS and correlate this to behavioral outcome.

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

References

1. Cardin, J.A., et al., *Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of Channelrhodopsin-2.* Nat Protoc, 2010. **5**(2): p. 247-54.
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6.

DEC-advies PV-2015-014-[redacted]

A. Algemene gegevens over de procedure

1. Aanvraagnummer; 2015-014

   Titel van het project; *Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease.*

2. Titel van de NTS; *Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease.*

3. Type aanvraag:

   X nieuwe aanvraag projectvergunning

   ☐ wijziging van vergunning met nummer

4. Contactgegevens DEC:

   - naam DEC; DEC-UM
   - telefoonnummer contactpersoon; [redacted]
   - mailadres contactpersoon; [redacted]

5. Adviestraject (data dd-mm-jjjj):

   X ontvangen door DEC; 08-03-2016

   ☐ aanvraag compleet

   X in vergadering besproken; 18-03-2016

   ☐ anderszins behandeld

   ☐ termijnonderbreking van 24-03-2016 tot 30-03-2016 /12-04-2016 tot 13-04-2016

   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen

   ☐ aanpassing aanvraag

   ☐ advies aan CCD

6. Eventueel horen van aanvrager **NVT**

7. Correspondentie met de aanvrager:

- Datum 24-03-2016

Strekking van de vragen:

1) **3.1:** U geeft aan te werken met 5-HT cre muizen. Desalniettemin ligt uw focus nergens op de raphe nucleus, waar de 5-HT cellen zich bevinden. Kunt u toelichten waarom u deze muizen wil gebruiken?
2) **3.4.2:** De muisexperimenten zijn helder opgeschreven en ook de modellen die gebruikt gaan worden zijn state-of-the-art. De DEC-UM vraagt zich echter af hoe de vraagstellingen 1, 2, en 3 worden vertaald met de literatuur (bv. wat is er bekend over DBS in muizen) – en hoe vertaalt zich de overstap naar ratten, beschreven in deelvraag 4?
3) **3.4.2:** Zijn de muismodellen geschikt om acute effecten van DBS op te pikken met deze reporter cellen?
4) **3.4.2:** In hoeverre is het bekend dat DBS acuut gezien al gunstige effecten heeft die aannemelijkerwijze niet zullen samenhangen met neurotransmitter switchen?
5) **3.4.3:** Er is een goed verband tussen de verschillende experimentele vraagstellingen. Deelvraag 1 bepaalt welk neurotransmitter systeem (of systemen) verder bestudeerd gaan worden in 2 en 3. Alle genetische muismodellen zijn voorhanden, maar hoeven dus niet allemaal te worden gebruikt. Aim 3 zal niet worden uitgevoerd als onder 1 geen positief resultaat wordt behaald. Ook hier geldt; deelvraag 4 is een onafhankelijk experiment en de vraag is: is dit een aparte studie, omdat dit in een ander diermodel wordt uitgevoerd en omdat dit los staat van deelvragen 1, 2 en 3?

3.4.4-appendix 1:

6) Er worden heel veel tests aangehaald waarvan max. 5 per dier worden gebruikt. Zijn dit altijd dezelfde tests en op basis van welke criteria worden deze gekozen? Kunt u misschien al specifieker aangeven welke tests u wilt gaan doen?

7) Er worden radiotracers gebruikt voor PET-CT scans. Zijn er pilotdata dat deze met voldoende resolutie/ specificiteit iets kunnen zeggen? Is dit haalbaar (resolutie PET is ~180um)?

8) De DEC-UM wenst een betere onderbouwing voor de gevraagde aantallen, alsmede een overzicht van de diergroepen, experimenten en berekening voor de aantallen.

9) In het hele document spreekt u van “human endpoints”. De DEC hoopt dat u 'Humane endpoints' bedoelt?

10) PD komt vrijwel niet voor bij mensen jonger dan 50 jaar. Reden voor het alleen gebruiken van mannen is dat vrouwelijke hormonen de hersenen veranderen. Waarom gebruikt u niet oudere ratten (vanaf 15-20 weken in plaats van 10-13 weken) en dan beide geslachten, zodat u een betere weerspiegeling krijgt van de patiëntengroep?
Als DBS een effect heeft op verandering van neurotransmitters, dan willen we dat toch voor zowel mannen als vrouwen weten?
U heeft niets vermeld over de leeftijd van de gebruikte muizen. Wellicht kunt u hier ook specificeren vanaf welke leeftijd u muizen in experiment wilt nemen en kunt u heroverwegen om zowel mannen als vrouwen te gaan gebruiken.

11) De DEC-UM vraagt zich af of er in de microcircuits veranderingen optreden met de leeftijd?

12) F: Accommodation and care: hierin schrijft u dat u niet voldoet aan Annex III of the Directive 2010/63/EU. U bedoelt waarschijnlijk dat u dit wel doet?

13) 3.4.4-appendix 3: Dit is een PD model in ratten, terwijl de rest van de experimenten in muizen gebeurt. Deelvraag 3 (MPTP model) gebruikt ook een PD model maar dan in muis. Zou dit niet gecombineerd kunnen worden?

- Datum antwoord 30-03-2016
- Niet alle vragen zijn naar tevredenheid beantwoord en de DEC-UM heeft dd. 12-04-2016 op nieuw een aantal vragen gesteld.
- De vragen zijn dd. 13-04-2016 naar tevredenheid beantwoord en hebben geleid tot aanpassing van de aanvraag.

8. Eventuele adviezen door experts (niet lid van de DEC) **NVT**

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet); **JA**
2. De aanvraag betreft een **nieuwe aanvraag.**
3. De DEC is competent om hierover te adviseren; **JA**
4. Vanwege betrokkenheid bij het betreffende project is een aantal DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, niet betrokken bij de advisering **NVT**.

C. Beoordeling (inhoud):

1. Het project is:

   X uit wetenschappelijk oogpunt verantwoord
   ☐ uit onderwijskundig oogpunt verantwoord
   ☐ uit het oogpunt van productiedoeleinden verantwoord
   ☐ wettelijk vereist

2. De in de aanvraag aangekruiste doelcategorieën zijn in overeenstemming met de hoofddoelstellingen.
3. De DEC onderschrijft het belang van de doelstelling. Het wordt ingeschat als

een substantieel belang.

4. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project: **JA.**
5. Er is sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren. De keuze hiervoor is voldoende wetenschappelijk onderbouwd. **NVT**
6. Het ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd: **JA.**
7. Er zijn geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen **vervangen.**
8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de vermindering van dierproeven. Het maximale aantal te gebruiken dieren is realistisch ingeschat. Vermindering wordt ondermeer verkregen door een powerberekening te hanteren, gebaseerd op resultaten uit het verleden en door een gefaseerd design toe te passen.

9. 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. Er is geen sprake van belangwekkende milieueffecten. Verfijning krijgt fraai vorm ondermeer door transgene diermodellen te kiezen die de ziekte van Parkinson het meest betrouwbaar weergeven en door minimaal invasieve benaderingen te hanteren.

10. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd

D. Ethische afweging

De DEC-UM heeft het project "*Neurotransmitter switching: A novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease"* bestudeerd. Het behelst terminale experimenten met matig ongerief gedurende 5 jaar met in totaal 800 transgene muizen. Anderzijds onderschrijft de DEC-UM de intrinsieke waarde van het dier.
De DEC-UM is overtuigd van de wetenschappelijke waarde en het uiteindelijk maatschappelijke belang van het voorgestelde onderzoeksproject.
Het project beoogt een beter begrip op te leveren van de werking van Deep Brain Stimulation (DBS) bij de behandeling van de ziekte van Parkinson (PD). Het gaat om het ontrafelen van de achterliggende neuronale mechanismen. Derhalve is het terecht

geclassificeerd als fundamenteel onderzoek. Aangezien DBS op dit moment wordt ingezet bij de behandeling van PD en wordt onderzocht bij andere aan de hersenen gerelateerde aandoeningen, kunnen de resultaten te zijner tijd ook klinisch relevant blijken. Het onderzoek kan zowel een beter begrip opleveren van therapeutische effecten als van eventuele bijwerkingen.
Resultaten van dit onderzoek zijn belangrijk voor PD patiënten en op termijn wellicht ook voor personen die lijden aan andere neurologische en psychiatrische ziekten en hun naasten. De DEC-UM acht derhalve de doelstelling van dit onderzoek van substantieel belang.
De opzet van het onderzoeksproject is helder, logisch en navolgbaar. De doelstellingen van de diverse onderdelen en de stapsgewijze aanpak, zijn overtuigend. De aanvrager beschikt over de benodigde wetenschappelijke kennis en technische expertise. Er is geen sprake van duplicatie. De gewenste uitkomsten zijn relevant in het licht van de overkoepelende vraagstelling en zijn ook haalbaar.
In de gekozen strategie, technieken en diermodellen wordt op bevredigende wijze tegemoet gekomen aan de vereisten op het gebied van vervanging, vermindering en verfijning. De DEC-UM is ervan overtuigd dat er geen alternatieven zijn, waardoor deze dierproef met minder muizen zou kunnen worden uitgevoerd, dan wel het gebruik van levende dieren zou kunnen worden vermeden.
Op grond van deze argumenten acht de DEC-UM in haar ethische afweging het belang van project 2015-014 "*Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease"* van zwaarder gewicht dan de voorziene schade (matig ongerief en dood) voor de maximaal 800 betrokken dieren. De DEC-UM beschouwt de voorgestelde dierproeven derhalve als ethisch gerechtvaardigd en voorziet het projectvoorstel "*Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease"* van een positief advies.

E. Advies

1. Advies aan de CCD

   X De DEC adviseert de vergunning te verlenen

2. Het uitgebrachte advies is gebaseerd op consensus.

Op grond van alle voor de afweging relevante argumenten komt de DEC-UM tot de conclusie dat dit onderzoek ethisch toelaatbaar is.

Centrale Commissie Dierproeven

7.

> Retouradres Postbus 20401 2500 EK Den Haag

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0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD107002016543

Bijlagen
2

Datum 12 mei 2016
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 10 mei 2016.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD107002016543. 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).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	10700
Naam instelling of organisatie:	Universiteit Maastricht
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	50169181
Straat en huisnummer:	Minderboedersberg 4-6
Postbus:	616
Postcode en plaats:	6200 MD MAASTRICHT
IBAN:	NL04INGB0679510168
Tenaamstelling van het rekeningnummer:	Universiteit Maastricht

Gegevens verantwoordelijke onderzoeker	
Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens plaatsvervangende verantwoordelijke onderzoeker

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

Gegevens verantwoordelijke uitvoering proces

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

Over uw aanvraag

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

Over uw project

Geplande startdatum:	1 maart 2016
Geplande einddatum:	1 maart 2021
Titel project:	Neurotransmitter switching a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease
Titel niet-technische samenvatting:	Neurotransmitter switching a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease
Naam DEC:	DEC Um
Postadres DEC:	Postbus 616, 6200 MD Maastricht
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.187,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

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

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Maastricht
Datum:	9 mei 2016

Centrale Commissie Dierproeven

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Universiteit Maastricht
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Onze referentie
Aanvraagnummer
AVD107002016543

Bijlagen
2

Datum 12 mei 2016
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 12 mei 2016
Vervaldatum: 11 juni 2016
Factuurnummer: 16700543

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD107002016543	€ 1.187,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL41RBOS 056.999.6317 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

8.

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD107002016543

Uw referentie

Bijlagen

Datum 7 juni 2016
Betreft Aanvulling Aanvraag projectvergunning dierproeven

Geachte [redacted]

Op 11 mei 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project 'Neurotransmitter switching: a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease' met aanvraagnummer AVD107002016543. 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:

- In de bijlage dierproeven geeft u aan 25 dieren/groep te gebruiken en een maximum aantal 400 dieren voor elke dierproef nodig te hebben. Echter ontbreekt de uitleg van de berekening van de 400 dieren en de beschrijving van de experimentele groepen. We verzoeken u om de berekeningen te verduidelijken, en het aantal groepen te benoemen en te onderbouwen.

- De titel van uw Niet-technische samenvatting is in het Engels doorgegeven. Omdat de NTS voor het publiek bedoeld is, moet de informatie die daarin komt in het Nederlands zijn. We verzoeken u om de titel in het Nederlands in te vullen en een nieuwe NTS naar ons toe te sturen.

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Gebruik hierbij het formulier dat u bij deze brief krijgt indien u uw antwoord per post verstuurt. Om uw aanvraag in de eerstkomende vergadering te kunnen bespreken verzoeken we u vriendelijk om uiterlijk maandag 13 juni 2016 uw antwoord naar ons toe te sturen.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat uw aanvraag compleet is. 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.

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

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Centrale Commissie Dierproeven

Melding

Bijlagen via de post

- 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

1.1 Vul de gegevens in.

Naam aanvrager			
Postcode		Huisnummer	

1.2 Bij welke aanvraag hoort de bijlage?
Het aanvraagnummer staat in de brief of de ontvangstbevestiging.

Aanvraagnummer	

2 Bijlagen

2.1 Welke bijlagen stuurt u mee?
Vul de naam of omschrijving van de bijlage in.

☐	
☐	
☐	

3 Ondertekening

3.1 Onderteken het formulier en stuur het met alle bijlagen op naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Naam	
Datum	- - 20
Handtekening	

Centrale Commissie Dierproeven

11.

> Retouradres Postbus 20401 2500 EK Den Haag

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Onze referentie
Aanvraagnummer
AVD107002016543

Bijlagen
1

Datum 27 juni 2016
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 10 mei 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Neurotransmitter switching a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease " met aanvraagnummer AVD107002016543. Wij hebben uw aanvraag beoordeeld.

Op 16 juni en 28 juni 2016 heeft u uw aanvraag aangevuld. U heeft de titel van uw Niet-technische samenvatting naar het Nederlands vertaald en aangepast.

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 voorwaarde betreffende het afstemmen van de go/no-go momenten met de IvD is gesteld in het kader van de 3V's, om te voorkomen dat dat dieren onnodig worden gebruikt in het geval dat de experimenten niet de verwachte resultaten opleveren. De algemene voorwaarde betreffende artikel 10, lid 1a van de wet wordt gesteld bij vergunningen met een langere looptijd. Dit om te voldoen aan datgene wat volgt uit dit artikel. U kunt met uw project "Neurotransmitter switching a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease " starten. De vergunning wordt afgegeven van 30 juni 2016 tot en met 1 maart 2021.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC Um gevoegd. Dit advies is opgesteld op 10 mei 2016. 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 1 juni 2016 heeft de DEC gereageerd op onze vragen. De DEC heeft het antwoord van de aanvrager op een door de DEC gestelde vragen naar de CCD gestuurd.
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. In aanvulling op het advies van de DEC stelt de CCD twee algemene voorwaarden.
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).

Met vriendelijke groet,

Centrale Commissie Dierproeven
namens deze:

[redacted]
Algemeen Secretaris

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

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Universiteit Maastricht
Adres:	Postbus 616
Postcode en plaats:	6200 MD MAASTRICHT
Deelnemersnummer:	10700

deze projectvergunning voor het tijdvak 30 juni 2016 tot en met 1 maart 2021, voor het project "Neurotransmitter switching a novel mechanism behind the therapeutic and side effects of DBS in Parkinson's Disease " met aanvraagnummer AVD107002016543, volgens advies van Dierexperimentencommissie DEC Um. De functie van de verantwoordelijk onderzoeker is Professor Neurochirurgie. Voor de uitvoering van het project is Postdoc verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 10 mei 2016
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 11 mei 2016;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 27 juni 2016;
c Advies van dierexperimentencommissie d.d. 10 mei 2016, ontvangen op 11 mei 2016.
d De aanvullingen op uw aanvraag, ontvangen op 16 juni en 28 juni 2016

Naam proef	**Diersoort/ Stam**	**Aantal dieren**	**Ernst**	**Opmerkingen**
3.4.4.1 Investigating the extent of transmitter respecification before and after DBS in an animal model of PD.	Muizen (Mus musculus) / Glu-Cre; TH-Cre; GABA-Cre; 5HT-Cre.	400	100,00 % Matig	
3.4.4.2 Studying the impact of this transmitter respecification on behavioral parameters in an animal model of PD.	Muizen (Mus musculus) / Glu-Cre; TH-Cre; GABA-Cre; 5HT-Cre.	400	100,00 % Matig	

Voorwaarden

Op grond van artikel 10a1 lid 2 Wod zijn aan een projectvergunning voorwaarden te stellen

In dit project worden dierproeven toegepast waarbij en wordt daarom voorzien van beoordeling achteraf. Deze beoordeling zal uiterlijk plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst de dierproeven conform de vergunning waren.
De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

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

Weergave wet- en regelgeving

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