		Inventaris Wob-verzoek W17-07								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 2016785**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Aanvraagformulier				x		x	x	
2		NTS	x							
3		Project proposal			x					
4		bijlage animal procedure 1			x					
5		bijlage animal procedure 2			x					
6		bijlage animal procedure 3			x					
7		bijlage animal procedure 4			x					
8		aanvullende informatie				x		x	x	
9		DEC advies				x		x	x	
10		Advies CCD aan bestuur		x						x
11		Beschikking				x		x	x	
12		herziene beschikking				x		x	x	

Centrale Commissie Dierproeven

20 DEC. 2016

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

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

1 Gegevens aanvrager

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

☒ Ja > *Vul uw deelnemernummer in* 10700 / 785

☐ 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 - 2 - 2017
Einddatum	1 - 2 - 2022

3.2 Wat is de titel van het project?

Deep brain stimulation to restore memory loss

3.3 Wat is de titel van de niet-technische samenvatting?

Diepe hersenstimulatie bij geheugenverlies

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, 6200 MD Maastricht
E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1584,00 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	[redacted]
Plaats	Maastricht
Datum	14 - 12 - 2016
Handtekening	[redacted]

Centrale Commissie Dierproeven

3

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.	Maastricht University
1.3	Provide the title of the project.	Deep brain stimulation to restore memory loss

2 Categories

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

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

3 General description of the project

3.1 Background

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

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

Deep brain stimulation (DBS) is a frequently used treatment alternative for various neurological and psychiatric disorders. In DBS, electrodes are stereotactically implanted in a target area of the brain and then connected to an implanted pulse generator. Attenuation of disease symptoms is then achieved by delivering an electric current to the targeted neuronal network. Currently, DBS is successfully used in the

treatment of drug-refractory movement disorders such as Parkinson's disease. The relatively low incidence of treatment-induced permanent neurological damage, reversible nature of the technique and possibility of tailoring stimulation parameters has proven this technique safe and patient-friendly. As such, DBS has become a valuable addition to common, generally more invasive, neurosurgical techniques. Subsequently, a growing number of psychiatric applications are being investigated for DBS, including depression and obsessive-compulsive disorder [1]. Recently, DBS has been suggested to be a promising new treatment modality for Alzheimer's Disease (AD) [2].
Evidence from recent clinical case studies suggests that DBS might enhance memory functions, when particular areas in the brain are stimulated [3]. In a single-case study, DBS was performed to treat a patient with morbid obesity, but unexpectedly stimulation evoked detailed autobiographical memory events. In the same year Vignal and coworkers showed that hallucinations of autobiographic memory could be evoked by stimulation of the amygdala, hippocampus and parahippocampal gyrus in epilepsy-patients [4].
Following these serendipitous findings, a few studies have tried to stimulate structures of the so-called memory circuit of the brain and have found beneficial effects when applying DBS correspondingly to the fornix [3, 5-7] or the entorhinal cortex [8, 9]. These structures are all directly connected to the hippocampus. Attention has also been drawn to the nucleus basalis of Meynert as a potential target structure for DBS in AD [10], since it has wide projections to the neocortex and the hippocampus. In the pathogenesis of AD the nucleus basalis of Meynert degenerates, leading to decreased cholinergic transmission and ultimately to cognitive decline in patients.
An important issue that needs to be raised is that up to now, most DBS studies in psychiatric disorders were first conducted in humans. In contrast to the application of DBS in Parkinson's disease, its use in AD is clearly short in preclinical evidence [11]. It is generally accepted that this therapy can be improved by obtaining knowledge of the optimal stimulation paradigms, the involved neuronal networks, DBS target areas and neurophysiological responses to acute and chronic DBS [12]. In our previous published studies [13], we observed that we can mimic both the therapeutic and side effects of DBS in animal models of various neurodegenerative diseases. This has led to new insights in to the neuronal networks affected by DBS and the mechanism of action [14]. Similar to recent clinical developments of DBS, we are evaluating DBS in animal models of other neurological and psychiatric disorders. With the studies proposed here, we aim to increase the therapeutic effect and decrease side effects of DBS by characterizing the optimal stimulation paradigm, DBS target area and desired neurophysiological response in an animal model. The primary outcomes are therefore the cognitive effects and behavioral side effects of different DBS stimulation paradigms. To identify the neuronal networks, brain regions and neurotransmitters involved, we will use intracranial sampling and recording methods, as well as imaging methods. Based on these first studies, we also want to investigate whether DBS is disease-modifying. For this we plan an experiment with a transgenic AD model. Lastly, we want to examine whether the effects of DBS can be enhanced by using drug treatments. The choice for these drug treatments is dependent on the underlying mechanism of DBS. However, considering standard medication for AD, we predict it will be modulators of the glutamate and cholinergic neurotransmitter systems, such as Memantine, Donezepil, Rivastigmine, Galantamine or similar.

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?

Based on the previous results of DBS in demented patients and studies in animal models of dementia, we hypothesize that DBS influences neuronal networks that are associated with cognition, mood and anxiety. In the current proposal, we address this hypothesis in four studies. First, we make an inventory of the cognitive effectiveness and occurrence of behavioral side effects upon DBS comparing the fornix to the nucleus basalis of Meynert within the memory circuit using different stimulation paradigms. Secondly, we evaluate the mechanism of action at the level of neuroanatomical network dynamics and neurophysiology. Thirdly, we want to investigate whether DBS is disease-modifying. For this we plan to conduct experiments with a model of disease. Finally, we investigate if the efficacy of DBS can be enhanced by drug treatments in our AD model. To meet these goals, we defined the following research objectives:

1. Define the cognitive and side effects of DBS in different stimulation paradigms.
2a. Analyse neurotransmitter changes induced by acute and chronic DBS.
2b. Identify acute and chronic DBS-induced changes in brain activity.
3. Define whether DBS can be disease-modifying.
4. Identify a drug treatment that increases the cognitive effect of DBS in a model of disease.

The above described research objectives may help to improve current DBS treatment of AD and reduce side effects. The feasibility of the proposed experiments is warranted as the involved research group has extensive experience with the surgical procedure of DBS, behavioral testing, brain imaging and intracranial sampling and recording methods as illustrated by publications. We expect to complete these studies within 5 years.

3.3 Relevance

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

Today, more than 44.4 million people suffer from dementia [15]. This number will increase to about 135.5 million in 2050. Every year 7.7 million new cases of dementia are diagnosed, implying that there is a new case of dementia somewhere in the world every four seconds. Dementia has emerged as one of the leading health problems of our time and has been recently recognized as one of the major threats to world population [16]. Symptoms include progressive loss of memory, impaired reasoning and judgement, difficulties paying attention, communication problems and various non-cognitive symptoms, ultimately leading to disability and need for care.
Most individuals diagnosed with dementia are 65 years or older, although there is a growing awareness of cases that start before the age of 65 [15]. Demographic ageing is a worldwide process resulting from constantly improving health care systems. The fastest growth in the elderly population is taking place in China, India, and their south Asian and Pacific neighbors. In 2010, the total estimated worldwide cost of dementia was US$604 billion, which equals to around 1% of the world's gross domestic product [17]. About 70% of the costs occur in Western Europe and North America.
Unfortunately, despite decades of research, we are still in need of an effective therapy for dementia, symptomatic or curative. There are 4 approved drugs on the market, which either modulate the cholinergic system by inhibiting acetylcholinesterase or reduce glutamate by antagonizing specific glutamate receptors [18]. These pharmacological interventions, however, have limited efficacy and severe-side effects for patients; therefore, we are in need of new, effective, and safe alternative treatment options.
Recently, deep brain stimulation (DBS) has shown to have beneficial effects across memory and cognitive networks. A first evidence for this emerged when Hamani and colleagues stimulated the fornix/hypothalamus area in a patient suffering from morbid obesity [3]. In this specific case, DBS generated detailed autobiographical memories in the patient. Based on this case-observation, the same group performed a phase-I trial in which six patients with mild Alzheimer's Disease (AD) were implanted with electrodes in the vicinity of the fornix [6]. After an intraoperative evaluation of stimulation to survey for recollective experiences and adverse effects, patients received chronic high frequency DBS for a period of 12 months (3.0–3.5 V, 130 Hz and 90 μs pulse width). The authors have found that the application of DBS in the hypothalamus/fornix vicinity was safe and triggered neural activity in the memory circuit, including the entorhinal and hippocampal areas. PET scans showed an early and striking reversal of the impaired glucose utilization in the temporal and parietal lobes that was maintained after 12 months of continuous stimulation. Evaluation of the Alzheimer's Disease Assessment Scale cognitive subscale and the Mini Mental State Examination suggested possible improvements and slowing the progression of memory loss at 6 and 12 months, especially in patients that were less severely affected at the time of surgery. In fact, 2 out of 6 patiens showed cognitive improvements, 1 patient remained stable and the other 3 deteriorated. Indeed, our presented project proposal is partly build upon this study. Because the clinical results were inconclusive, we feel that it is important to investigate the effects of DBS on the fornix with a variety of stimulation parameters and also to examine neurochemical and neurophysiological responses. Because, despite the encouraging results of the clinical trials presented above, basic neural and chemical mechanisms underlying DBS are still debated [19] and one approach to address these issues is to investigate the effects by stimulating homologous regions in experimental

animal models [12].
Therefore, the studies described in this proposal aimed at investigating which DBS target structures and stimulation parameters produce the most beneficial effects in an experimental model of dementia. In addition, the present proposal also aimed at investigating potential mechanisms of action of DBS with regard to memory restoration. Only through understanding the mechanisms, DBS therapy in dementia patients can be fine-tuned to produce the best possible symptom relief currently available.

3.4 Research strategy

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

The first goal is to compare the cognitive effect of different DBS stimulation paradigms and characterize the side effects on behavior. To achieve this, we stereotactically implant electrodes in the fornix or nucleus basalis of Meynert to apply DBS. EEG electrodes are implanted in the hippocampus in order to measure changes in theta rhythm. After implantation, DBS animals will be subjected different stimulation parameters while sham animals are only attached to cables and not stimulated. Their behavior will be evaluated in several tests. Memory impairment is induced pharmacologically by injecting scopolamine intraperitoneally 30 min before behavioral testing. Also proliferative cells will be labeled during this study and the effects on neurogenesis will be evaluated. The second goal is to evaluate the mechanism of action on the level of neuronal networks, brain regions and neurotransmitters. To evaluate neurotransmitter and neurophysiological responses of brain regions, we will use intracranial sampling (microdialysis to measure neurotransmitter levels; optical fiber probes for fiber photometry to measure brain activity), EEG recording (electrophysiology) and imaging methods (PET-CT imaging). We will evaluate these responses in both, a terminal acute DBS experiment and a chronic DBS experiment. Acute experiments will be performed during surgery or immediately afterward and will give insights to immediate mechanisms of action of DBS. Chronic experiments are conducted after the complete restoration of the physiological functions that were altered by anesthesia or surgery and will provide insights to the long-term effects of DBS in the brain. The third goal is to evaluate if DBS is disease-modifying. For this, we plan to conduct experiments in an AD rat model. Finally, we want to investigate whether cognitive effects can be enhanced in this AD rat model by using drug treatments. We will compare the effect of different drug treatments combined with DBS on the cognitive effect, generation of side effects and neurotransmitters and neurophysiological responses. To evaluate this, we will use a selection of the behavioral tests, intracranial sampling/recording methods and imaging methods used in the first studies of the project.

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.

This project consists of four studies. Our group has accumulated sufficient experiences with all procedures described below [5, 13, 14, 20-23].

Study 1 - The effects of DBS on cognition, behavior and neuronal networks. Animals are first subjected to a baseline measurement of neuronal networks using preclinical imaging methods under general anesthesia, subsequently brain electrodes are implanted as described below in the DBS procedure. In order to measure cognitive effects with DBS, memory impairment is pharmacologically-induced for the duration of the behavioral tasks. Next, animals are subjected to behavioral tests centered around memory, cognition, mood and anxiety in DBS stimulation on and off periods. The cognitive effect will also be indicated by labelling proliferative cells in order to assess neurogenic changes. After behavioral testing, electrodes will be removed and the effect of DBS on neuronal networks will be assessed by brain imaging identical to the baseline measurement. At the end of the experiment, all animals are euthanized.

Study 2 - Changes in neurotransmitters and brain activation induced by acute and chronic DBS. Animals are subjected to the same surgical procedure for implantation of the DBS electrodes. During surgery intracranial sampling probes for neurotransmitter sampling (microdialysis) and measuring brain activity (fiber photometry) are placed in the hippocampus.
Animals that receive acute DBS will only be stimulated for a short period of time under general

anesthesia. During this session, intracranial sampling and recording probes are used to assess the changes in neurotransmitters and brain activation induced by acute DBS. At the end of this session, animals will be sacrificed.
Animals that receive chronic DBS undergo the same surgical procedure for DBS electrode and hippocampal probe implantation, and are left to recover as described in the DBS procedure below. Similar to study 1, memory impairment is pharmacologically-induced for the duration of the behavioral task and these animals are then subjected to behavioral tests with DBS stimulation on and off. Subsequently, in freely moving rats, intracranial sampling probes are used to assess the changes in neurotransmitters and brain activation induced by chronic DBS. Subsequently animals will receive general anesthesia and will be subjected to intracranial electrophysiology recording, fiber photometry or PET-CT brain imaging. Afterwards, all animals will be euthanized.

Study 3 – This experiment is only carried out in case we can restore memory loss by DBS in study 1 using specific stimulation parameters. Study 3 is a long-term DBS study in a Alzheimer model of disease in order to define whether DBS is disease-modifying. The primary goal of this study is to see if DBS has a positive effect on brain pathology in AD. DBS electrodes and hippocampal probes are implanted by stereotactic brain surgery. Subsequently, behavioral tests, intracranial sampling and electrophysiological recordings which we will derive from study 2, will be carried out. Afterwards, all animals will be euthanized.

Study 4 - This experiment is also only carried out in case we can restore memory loss by DBS in study 1 using specific stimulation parameters. Study 4 investigates the role of neuropharmacology in DBS for dementia. This study is performed to see if we can further enhance memory through combining DBS with neuropharmacology. Animals are subjected to the same surgical procedure for implantation of the DBS electrodes as described below and in study 1-2. Thereafter, animals are treated with a selection of drugs or receive a placebo. Similar to study 1, animals will be subjected to behavioral tests and DBS stimulation on and off periods to evaluate the cognitive effect and generation of side effects. Intracranial sampling and recording probes are used to study the underlying mechanism, similar to study 2. Afterwards, all animals will be euthanized.

*DBS procedure: Animals will undergo stereotactic surgery for electrode implantation. Under general anesthesia, rats will be implanted with DBS electrodes [23] bilaterally in the fornix or nucleus basalis of Meynert and will receive one additional electrode in the hippocampus. The electrodes will be fixed on the skull. The electrodes are connected to an external pulse generator for stimulation and EEG recordings through the hippocampal electrode in freely moving rats. After surgery, animals will receive a recovery period of at least 1 week. Following this, behavioral testing is performed with DBS. Sham animal will undergo the same surgical procedure, but are not stimulated through the electrodes. Depending on the behavioral test paradigm animals are additionally trained before surgery. Stimulation can be performed at different parameters such as low and high frequency stimulation (20 to 130 Hz) with various amplitudes (50 to 500 µA) and pulse widths up to 100 µs [24]. Please note, for testing various stimulation parameters, we included a minimum of 24h stimulation-off period for all animals.

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

In this project, we aim to define the cognitive effects and behavioral side effects of DBS in different stimulation paradigms, underlying mechanisms and our ability to influence this mechanism with DBS and neuropharmacology. To illustrate the effect of DBS on the brain we provide information on several coherent levels. We will provide information on the cognitive effect, information on behavioral side effects, and information on underlying mechanism of neuronal networks, neurotransmitters and brain regions. We will also study the additional influence of different drug treatments. This multimodal project will investigate the effects of DBS from the level of behavior to neurotransmitter and back again. This project entails the following selection points and milestones. The timeline of this research project is summarized in Figure 1 while the Go-NoGo criteria are summarized in Figure 2. Since fornix and nucleus basalis of Meynert DBS have been already applied experimentally in the clinics with inconclusive results [6, 10], we will explicitly perform study 1 and 2, in order to elucidate on optimal stimulation protocols or

underlying acute and chronic mechanisms (i.e. all experimental groups in study 1 and 2 will be used). Refinement of study 2 is achieved by only stimulating animals with the most optimal stimulation parameter derived from study 1. Study 3 and 4 are dependent on the results obtained in study 1 and 2 (non-significant findings in study 1 and 2 will lead to termination of the project). Moreover, only the DBS target showing restoration of memory loss in study 1 (fornix DBS vs. nucleus basalis of Meynert DBS) as well as the physiological brain response to DBS (neurotransmitter vs. brain activity) showing significance in study 2, will be used for study 3 and 4.

Study 1 – Selection of most optimal DBS target, behavioral tests and stimulation paradigm. In this study we will test if we are successful in restoring pharmacologically-induced memory loss with DBS in freely moving rats. For this, we will specify the most optimal stimulation paradigm for the subsequent studies.

Study 2 – Selection of intracranial sampling and recording methods, neurotransmitters and brain regions. In an acute and chronic DBS experiment, we will test which intracranial sampling and recording methods can display the underlying mechanism of DBS. The stimulation paradigm and behavioral tests used in this study are based on study 1.

Study 3 – DBS in a model of disease. We will investigate if DBS can also alleviate symptoms in a model of Alzheimer's. Our findings in study 1 and 2, will guide us in choosing a suitable model of disease for this study (e.g. animal model, in which brain pathology is accompanied by a decline of a certain neurotransmitter, brain activity etc.). Moreover, the stimulation paradigm, target area and behavioral tests used in this study are based on study 1 and 2.

Study 4 – Neuropharmacology. We will compare different drug treatments in conjunction with DBS. The choice of drugs is based on the results of the underlying mechanism evaluated by study 2. We will use the same intracranial sampling and recording methods that have reflected the underlying mechanism as investigated in study 2. The stimulation paradigm, target area and behavioral tests used in this study are also based on study 1 and 2

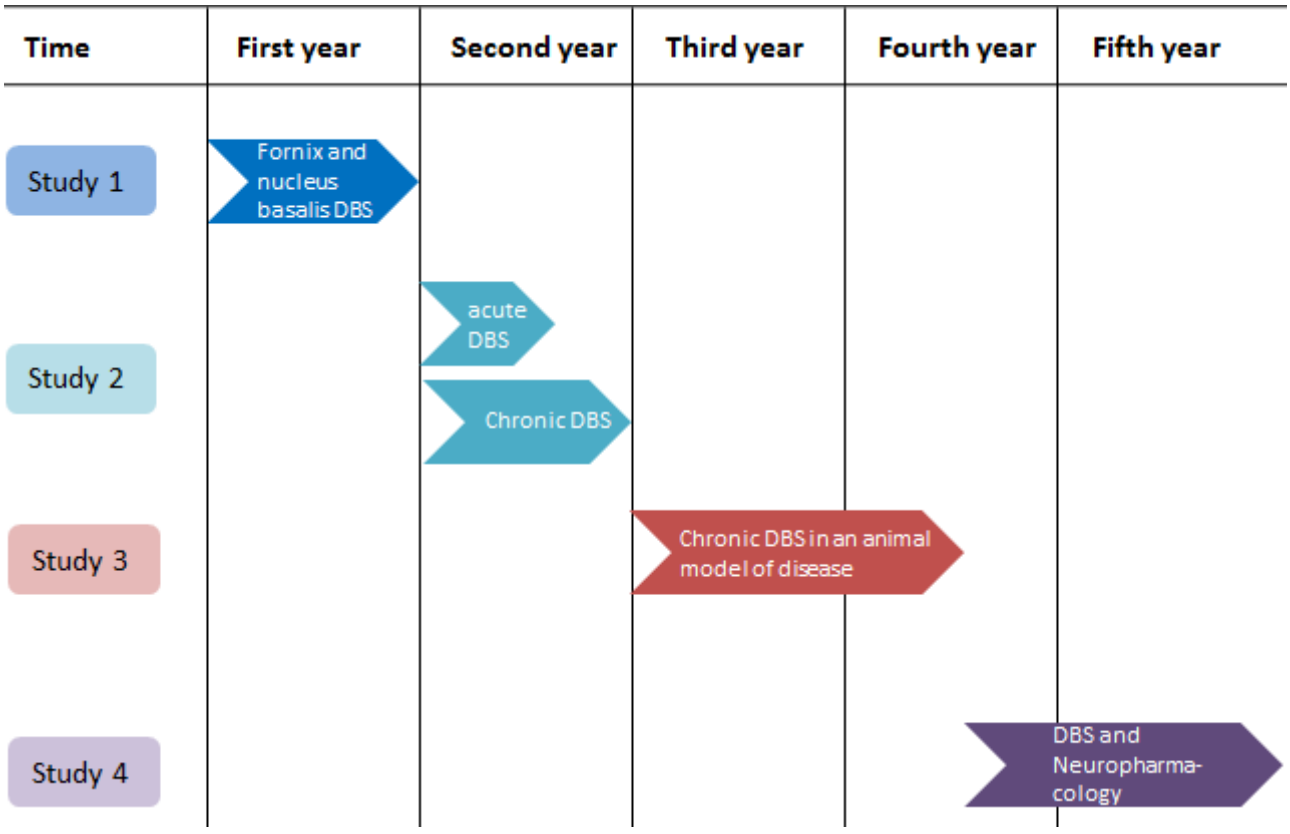

Figure 1. Summary of the studies and timeline of the research project. DBS, deep brain stimulation.

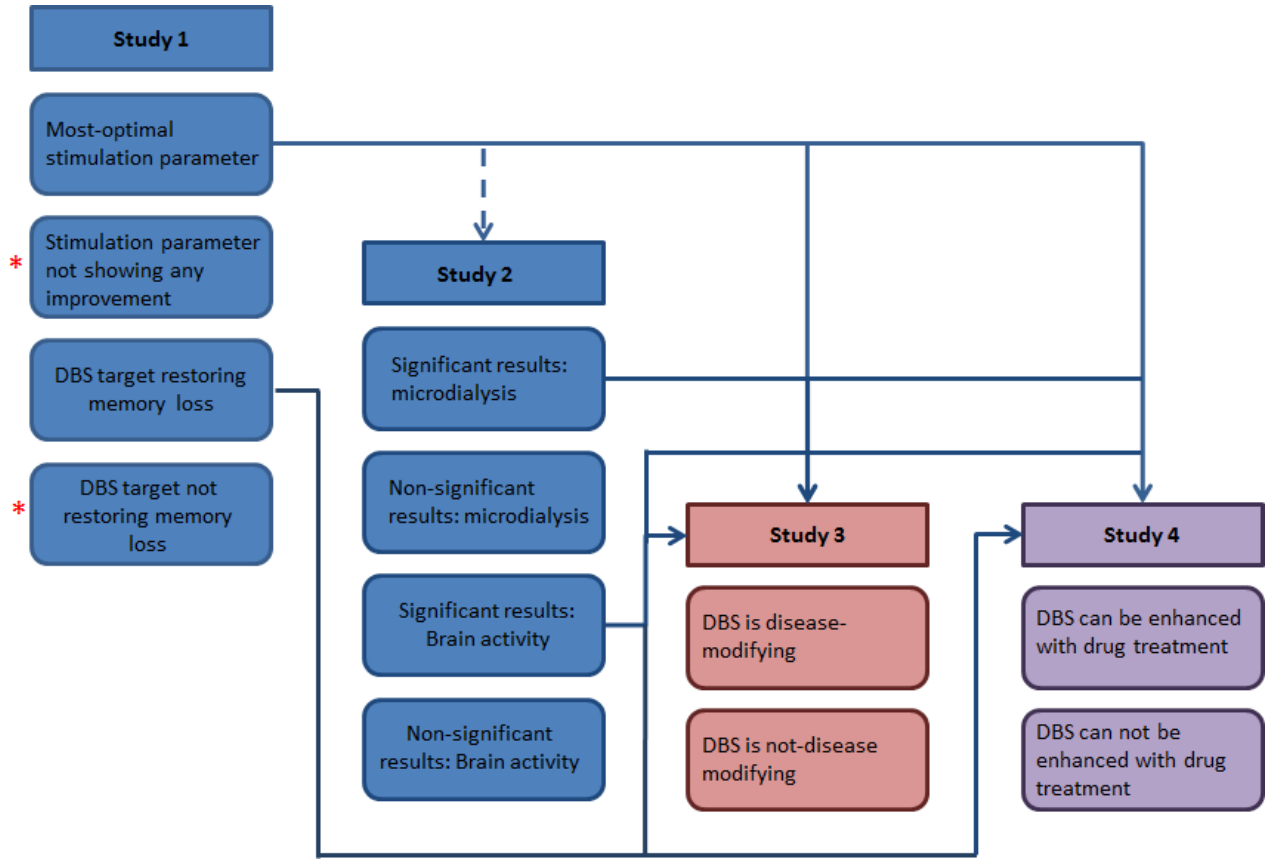

Figure 2: Go/No-go chart of project proposal. Arrows indicate "Go"-condition and * indicates a no-go condition for study 3 and 4. This means that study 1 and 2 will be carried out and only if DBS is able to restore memory loss with specific stimulation parameters, study 3 and 4 are conducted. In study 2, only the most-optimal stimulation parameter derived from study 1 is used. Microdialysis and brain activity results will provide insights in the possible mechanisms of action of DBS. Based on these findings we can select an appropriate animal model for study 3 and 4. Moreover, only the DBS target showing restoration of memory loss in study 1 (fornix DBS vs. nucleus basalis of Meynert DBS) as well as the physiological brain response to DBS (neurotransmitter vs. brain activity) showing significance in study 2, will be used for study 3 and 4.

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

Serial number	Type of animal procedure
1	The effects of fornix and nucleus basalis DBS on cognition, behaviour and neuronal networks
2	Changes in neurotransmitters and brain activation induced by acute and chronic fornix and nucleus basalis DBS
3	Long-term DBS in an animal model of disease
4	The role of neuropharmacology in DBS for dementia-related disorders
5	
6	
7	

8	
9	
10	

References

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Appendix
Description animal procedures

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- 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: 3.4.4.1 Type of animal procedure: The effects of fornix and nucleus basalis DBS on cognition, behavior and neuronal networks

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 the fornix and nucleus basalis of Meynert in an experimental model of memory loss, which is induced pharmacologically (e.g. intraperitoneal scopolamine injections). Consequently, we will evaluate the therapeutic effect (cognition) and side effects at different DBS settings. To identify neuronal networks that may exert these DBS effects, the intervention is preceded and followed by high resolution MR imaging.
The primary outcome of this study is to evaluate the cognitive effects and behavioral (side) effects of DBS. The readout parameter for the cognitive effect and behavioral (side) effects will vary per test and include cognition, memory, mood and anxiety. A summary of the possible different behavioral tests, readout parameters and justification is found in Table 1.
The secondary outcome of this study is to identify the neuronal networks that may exert behavioral (side) effects. The readout parameter for structural and functional connectivity of neuronal networks will entail the anatomic (re)organization of white matter tracts originating from the fornix or nucleus basalis of Meynert and resting activity related brain regions generated by diffusion tensor MRI imaging and resting state functional MRI, respectively. Moreover, proliferative cell labelling will provide insights to neurogenic processes in the hippocampus and EEG electrodes will allow us to evaluate changes in hippocampal theta rhythms during DBS on and off periods.

Though all animals will undergo behavioral tests, the selection of tests may change in the course of the study due to intermediate analyses or studies of other research groups, which were completed before ours. We will thus not use all behavioral tests as presented in Table 1, but will make a selection in the beginning of the study for follow-up studies (DBS in rats of appendix 2-4). The maximum number of

behavioral tests is 10 (5 for the domains cognition/memory and 5 for the domains anxiety/mood). The minimum number of behavioral tests is 6 (3 for each domain). However, we expect to further reduce the number of behavioral tests during the research project to a minimum of 4 and maximum of 7 behavioral tasks in appendix 2, 3 and 4. Behavioral tests that show a significant difference between the DBS on and off will be used in follow-up studies. If multiple behavioral tests are suitable and each could answer the research question of interest, we will select the test with the least degree of discomfort for following studies. Cumulative discomfort and contamination effects caused by repeated behavioral testing will be minimized by having a minimum of 24h between different tests and by starting with the behavioral task causing the least amount of discomfort (1, 2).

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

Test paradigm	Item	Readout parameter	Discomfort
Barnes maze	memory	number of errors per trial, rate of decline in number of errors and path length	Mild discomfort, results in increased anxiety
Morris water maze	memory	time spent in the platform quadrant, escape latency and swimming speed	Moderate discomfort, animals will be in water
Cued and contextual fear conditioning	memory	percentage of time spent freezing and time to extinction	Moderate discomfort, small electrical shock will be administered
Object location task	memory	ratio of time spent at object in new versus old location	Mild discomfort, results in increased anxiety
Object recognition task	memory	ratio of time spent at new versus old object	Mild discomfort, results in increased anxiety
Y-maze	memory	Ratio left-right discrimination	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
Prepulse inhibition	cognition	startle response	Moderate discomfort, animals will temporarily be in a restrainer
Forced swim task	mood	immobility duration, latency to immobility, swimming duration, climbing duration	Moderate discomfort, animals will be in water
Sucrose intake/preference	mood	volume of sucrose intake corrected for animal weight	Mild discomfort, results in increased anxiety
Food intake	mood	volume of food intake corrected for animal weight	Moderate discomfort, animals will receive restricted feeding regimes or deprivation
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 and locomotion	total distance travelled, average speed and time spent in closed arms	Mild discomfort, results in increased anxiety
Home-cage emergency	impulsivity and anxiety	latency to escape	Mild discomfort, results in increased anxiety

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 entails DBS, MR imaging, EEG recordings and behavioral tests in freely moving or anaesthetized rats (rats are merely anaesthetized for MR imaging). For a summary of the experimental groups see section 2B. The procedures proposed in this study are comparable to those published in literature (3, 4) and previous studies (5-7) in which we used similar imaging methods, DBS and behavioral testing paradigms in animal models for neurodegenerative diseases.

Main study
MRI – baseline
Animals are first subjected to a baseline measurement of neuronal networks using preclinical 7 Tesla MR brain imaging under general anesthesia. An imaging protocol of about 2 hours per animal is applied to obtain high resolution anatomical T2, diffusion tensor imaging, resting state functional MRI and spectroscopy information (8). Subsequently, animals return to their home cage for a minimum of 1 week and, depending on the behavioral paradigm, they are trained for maximally 2 months and then receive brain electrodes.

Surgery and behavioral experiments
DBS electrodes are bilaterally implanted in the fornix or nucleus basalis of Meynert and an EEG electrode is placed in the hippocampus. Sham animal will undergo the same surgical procedure, but are not stimulated. In the chronic phase, animals will be subjected to behavioral tests for memory, cognition, mood and anxiety. As mentioned in section 2A, the number and choice of behavioral test may vary in course of the study, but are set at maximally 10 in the first and 7 in following studies. Behavioral tests are performed in both DBS on and off conditions. Different experimental groups consist of animals that receive high (130 Hz) and low (20 Hz) frequency DBS in different stimulation paradigms (constant, random and EEG responsive stimulation) for various time durations (maximally 24 hours per day). To be able to evaluate the effect of DBS on newly integrated neuronal networks (e.g. hippocampal neurogenesis), that are not detectable by MRI, animals will receive injections with a label that detects proliferative cells (bromodeoxyuridine, maximum 2 times a day for 5 days). To be able to evaluate the effect of DBS on changes in hippocampal theta waves, which cannot be detected by MRI, animals will undergo EEG recordings.

MRI – end
After behavioral testing, the electrode construct is removed under general anesthesia and animals are subjected to the same MRI protocol as performed at baseline. Subsequently, animals are euthanized appropriately for post-mortem analysis of the brain. Due to the possibility that the MRI scanner may not be available at our university during the study, we will consider to perform this part of the study in a different Dutch or European center with a license to perform animal experiments. Possible locations entail the University of Leuven, Gent, Utrecht or Aachen. In that case we will file a working protocol and / or an ethical application at the local facility. Based on our previous experience, we estimate a maximal experimental time of 10 months per animal.

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

To estimate the number of animals needed, we considered published studies and previous studies by our group. To minimize the number of animals used, we will perform a power analyses.

B. The animals

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

Animals
These experiments require male adult rats purchased from a registered breeding facility, as we have routinely used these animals in our previous DBS studies (5, 6, 9).

Gender
We will only use male rats, since the oestrogen cycle can interfere with the behavioral outcome and brain biochemistry, such as release of the neurotransmitters dopamine and serotonin (10). Accounting for sex as a biological variable in all biomedical research is considered fundamental for enhancing rigor and reproducibility in preclinical research. Since we have behavioral and neurochemical outcome measures, we therefore want to reduce the variability in our data by using only male rats.

Number of animals
The estimation on the total number of animals is based on our previous experience with DBS studies and a literature review. To minimize the number of animals used, we will perform a power analyses to establish the total number of animals per group and take a possible drop out of animals into account. Based on our previous experience with DBS experiments, factors such as complication of surgery, electrode loss or incorrect electrode localization will lead to a dropout of animals. Within our research group, this dropout ranges from 10% to 25% and is dependent on the duration of the experiment and animal procedures during the experiment. We thus estimate a maximum dropout of 25% and aim to reduce this number during the research project by optimizing the surgical technique and animal procedures. Considering literature and our previous experience with DBS experiments, we estimate to need a maximum of 20 animals per group for naïve rats. We will investigate 4 groups in total (see Table 2).

Power analysis: Literature review and previous experience indicate a significant effect at $\delta=0.3$ and a standard deviation of $\sigma=0.3$. The significance level is $P < 0.05$ and we require a power of $\eta=0.8$. The readout parameter for the cognitive effect and behavioral (side) effects will vary per test and include cognition, memory, mood and anxiety. A summary of the possible different behavioral tests, readout parameters and justification is found in Table 1. The readout parameter for structural and functional connectivity of neuronal networks will entail the anatomic (re)organization of white matter tracts originating from the fornix or nucleus basalis of Meynert and resting activity related brain regions generated by diffusion tensor MRI imaging and resting state functional MRI, respectively. Moreover, proliferative cell labelling will provide insights to neurogenic processes in the hippocampus and EEG electrodes will allow us to evaluate changes in hippocampal theta rhythms during DBS on and off periods.

$N = 15.7 * (0.3/0.3)^2 = 15.7$

Due to factors such as complication of surgery, correct electrode locations and possible electrode losses, we estimate 25% of loss per group.

$N = 15.7 / 0.75 = 20.9$ i.e. 21 animals per group.

Table 2: Number of animals needed for study 1. Different stimulation paradigms are applied to the fornix and nucleus basalis of Meynert throughout the study. A stimulation off period of at least 24h will prevent carry-over effects as described here [5]. DBS, deep brain stimulation

Study	Group	Number of animals
1 (Induced memory loss-Chronic DBS)	Fornix DBS	21
	Nucleus basalis DBS	21
	Fornix sham	21
	Nucleus basalis sham	21

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 evaluate the cognitive effects and behavioral (side) effects of fornix and nucleus basalis of Meynert DBS. The secondary aim is to identify the neuronal networks that may exert behavioral (side) effects. 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 (4, 11) 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. Though dementia research is also conducted with lower animal species such as zebrafish, drosophila and caenorhabditis elegans, the use of these animals for DBS research has several major drawbacks or is simply impossible. Due to the small size of the animal's nervous system and current spread of DBS electrodes, we cannot selectively stimulate one brain area such as the fornix or nucleus basalis of Meynert, nor can we perform behavioral tests during stimulation. Additionally, the loss of complexity in neural networks by using a lower animal species will generate research results that are less likely to be translated to humans. The current study can also not be performed in humans because of the following: 1. DBS at different (experimental) stimulation paradigms is not ethical in humans, as it is unpredictable if they may be harmful (i.e., epileptic seizures) 2. High resolution MR imaging cannot be performed after the implantation of DBS electrodes due to technical constraints and safety issues and it is ethically not accepted to remove the electrodes in patients. 3. Sham implantations of electrodes are unethical.

Reduction
We will minimize the number of animals in this study by using a power analysis. Additionally, animal numbers are minimized by the optimal study design, experience with the pharmacological-induced memory impairment model, state-of-the-art EEG measurements, imaging equipment and optimized behavioral tests after several years of experience. Moreover, only competent and trained persons will carry out the surgical procedure.

Refinement
The first study of DBS in memory-impaired animals will serve to select the behavioral tasks to use in future experiments and will therefore help to minimize the number of behavioral tasks and thus reduce animal experiment time during the research project. Finally, we will adapt the care to the need of the animals at the different stages of the experiment (e.g. recovery-food during recovery period after surgery, cage enrichment in case animals have to be single-housed, etc). Only competent and trained persons will carry out the surgical procedure.

Explain what measures will be taken to minimise 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. Similarly, if animals show pain during any of the experimental procedures described, they will receive analgesics. To prevent the occurrence of more than predicted animal suffering, we will implement humane endpoints (see section J). 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.

N/A

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.

At the first instance we will house all animals in pairs to minimize distress. However, it is possible that rats may damage each other's electrode construct, which in turn could lead to injury, infection, and loss off the electrode construct. Consequently, this can cause a humane endpoint, euthanasia of the animal and a decrease of experimental power. This therefore introduces a consideration of animal welfare (Refinement) versus the Reduction principle of experimental animal ethics. In case only *one* rat pair damages each other's electrode construct, we will have to house *all* animals individually. However, to mitigate some of the negative consequences of individual housing, we will use enrichment objects (12).

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.

Location of MR imaging
Due to the possibility that the MRI scanner may not be available at our university during the study, we will consider to perform this part of the study in a different Dutch or European center with a license to perform animal experiments. Possible locations entail the University of Leuven, Gent, Utrecht or Aachen. In that case we will file a working protocol and / or an ethical application at the local facility.

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

Local rules of housing, care and treatment will apply.

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 and imaging procedures. Animals will be monitored daily during the experimental procedures stated in section 2A and will be checked for signs of pain or discomfort. Analgesics will be applied to relief suffering e.g. during the pre-, peri- and post-

surgical recovery period. Treatment of pain or suffering will be conducted according to the recommendations of GV-SOLAS: Pain management for laboratory animals (May 2015).

I. Other aspects compromising the welfare of the animals

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

Possible adverse effects which might occur are experience of stress.

Explain why these effects may emerge.

The electrode construct is cemented on the skull of the animal. In the postoperative period the headskin of the animal will heal and grow around the electrode construct. However loss of the electrode construct may still occur due to suboptimal healing of head skin around the construct or mechanical stress during behavioral testing. Animals might also experience stress due to injections of labelling proteins for newly born cells and brain stimulation might induce stress, tingling sensation and balance problems.

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

Animals will be visually inspected daily during experiments. Loss of an electrode construct during the postoperative period or behavioral testing is prevented by the extensive experience in behavioral testing and refinement of surgical techniques performed by our research group over the past 10 years. This has recently led to some detailed changes such as bending the DBS cables along the axis of the animal and using sufficiently high cages during behavioral testing. Additionally, completely drying the skull during stereotactic surgery and working aseptic will minimize electrode loss. Stress due to injections will be minimized by skilled injection techniques and handling of the animals. Animals that experience acute adverse effects caused by DBS during behavioral testing (e.g. seizure), will be released from the stimulation immediately (DBS turned off).

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.

Humane endpoints will be based on considerations regarding general health, severe weight loss (20% body weight loss when compared to the pre-study weight), infections, loss of electrode construct and animal behavior.

Indicate the likely incidence.

We estimate the likely incidence of implementing a humane endpoint at maximally 15%. In the course of this research proposal we will optimize surgical and animal procedures to reduce this incidence.

K. Classification of severity of procedures

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

-Behavioral tests: Moderate discomfort. See table 1.
-MRI imaging: mild discomfort, due to anesthesia and relocation of animals
-Stereotactic surgery: moderate discomfort due to surgery
-DBS and EEG measurements: mild discomfort due to new environment
-Possibility of individual housing: moderate discomfort due to non-social behavior
-Injections: mild discomfort, can result in increased stress/anxiety
-Euthanasia: mild discomfort, due to anesthesia

Cumulative discomfort: 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 euthanized 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

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Centrale Commissie Dierproeven

5

Appendix
Description animal procedures

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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: 3.4.4.2 Type of animal procedure: Changes in neurotransmitters and brain activation induced by acute and chronic fornix and nucleus basalis DBS

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 design** of this study is an **evaluation of DBS intervention**. We will evaluate the changes in neurotransmitters and brain activation induced by acute and chronic DBS.

The primary aim of this study is to identify the changes in neurotransmitters induced by acute and chronic DBS. The readout parameter will entail the change in neurotransmitter levels in the hippocampus during acute DBS under general anesthesia and chronic DBS during behavioral testing. To quantify the level of neurotransmitters we will use intracranial sampling methods such as microdialysis.

The secondary aim of this study is to identify the changes in brain activation induced by acute and chronic DBS. The readout parameter for brain activation will entail the change in local field potentials in the hippocampus measured by intracranial electrophysiology recordings and the activity change in specific brain regions measured by PET-CT imaging. We will also use fiber photometry in order to quantify region-specific changes in Calcium influx.

We justify the choice for these intracranial sampling and recordings methods, because in previous studies we have shown that these methods can identify DBS induced changes of neurotransmitter levels and brain region activation. We also expect DBS-related changes in Calcium influx in specific brain region targets like the hippocampus. This has led to the validation of several experimental animal models and new insights in to the mechanism of action of DBS in animal models for neurodegenerative diseases [1-

4].

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

Test paradigm	Item	Readout parameter	Discomfort
Barnes maze	memory	number of errors per trial, rate of decline in number of errors and path length	Mild discomfort, results in increased anxiety
Morris water maze	memory	time spent in the platform quadrant, escape latency and swimming speed	Moderate discomfort, animals will be in water
Cued and contextual fear conditioning	memory	percentage of time spent freezing and time to extinction	Moderate discomfort, small electrical shock will be administered
Object location task	memory	ratio of time spent at object in new versus old location	Mild discomfort, results in increased anxiety
Object recognition task	memory	ratio of time spent at new versus old object	Mild discomfort, results in increased anxiety
Y-maze	memory	Ratio left-right discrimination	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
Prepulse inhibition	cognition	startle response	Moderate discomfort, animals will temporarily be in a restrainer
Forced swim task	mood	immobility duration, latency to immobility, swimming duration, climbing duration	Moderate discomfort, animals will be in water
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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 and locomotion	total distance travelled, average speed and time spent in closed arms	Mild discomfort, results in increased anxiety
Home-cage emergency	impulsivity and anxiety	latency to escape	Mild discomfort, results in increased anxiety

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

This study is an acute and chronic DBS experiment that entails DBS, EEG registrations, behavioral testing and intracranial sampling and recording methods under general anesthesia and in freely moving rats during behavioral testing, followed by PET-CT imaging.

Acute DBS
This is a terminal experiment for acute DBS. DBS electrodes are implanted by stereotactic brain surgery (see appendix 1 and SOP 1). We will only use naïve rats and perform DBS or sham operation. In the same session under general anesthesia, we will insert the microdialysis, optical fiber or electrophysiology probes. In case of fiber photometry, a virus (AAV-GCamP) will be injected into the brain target 4 weeks prior to DBS surgery. Neurotransmitter sampling is performed in both DBS on and off stimulation settings. Since it is not possible to combine microdialysis with electrophysiology/viral delivery for fiber photometry, we will divide the animals into two groups: Neurotransmitter (NT) and brain activity (BA) group, respectively. Moreover, since this is an acute experiment to evaluate immediate brain responses to DBS, behavioral tests are not performed and thus no pharmacologically-induced memory impairment is needed. At the end of each experiment, rats are euthanized and the brain is removed for further histological analyses.

Chronic DBS
Similar to the study described in appendix 1, this is a chronic DBS experiment. We define chronic DBS as stimulation for maximally 24 hours per day for at least 4 weeks per animal [16] with a maximal experimental duration of 10 months. We will use naïve animals, whereas memory impairment will be induced pharmacologically for behavioral testing. DBS electrodes are implanted by stereotactic brain surgery under general anesthesia (see appendix 1). Additionally, intracranial sampling probes for fiber photometry or microdialysis are implanted. Similar to study 1, behavioral tests in DBS on and off conditions will be performed, but this time only the most optimal stimulation parameter derived from study 1 will be used. The expected minimum number of behavioral tests is 4 and the maximum number of behavioral tests is 7. Since this study investigates the mechanisms of action of the most optimal stimulation parameters for both DBS target structures, sampling of neurotransmitters will be performed during behavioral testing and calcium flux is measured through fiber photometry probes. Then, similar to the acute DBS experiment, electrophysiological recordings of brain activity are performed under general anesthesia in DBS on and off conditions. Half of the total number of animals is used for intracranial sampling recordings, the other half receive an intraperitoneal or intravenous injected tracer (tracer for different neurotransmitter systems will be defined based on the results of the acute experiment, or elsewise indicate glucose metabolism) for PET-CT imaging in DBS on and off conditions under general anesthesia. Subsequently, all rats are euthanized and the brain is removed for further histological processing.
Since this study contains a chronic DBS experiment similar to appendix 1, we estimate a maximal experimental duration time of 10 months per animal.

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

We have based the number of animals on literature review, previous studies performed by our research group and a power analyses. The power analyses is presented in section 2B.

B. The animals

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

Animals
These experiments require male adult rats purchased from a registered breeding facility, as we have routinely used these animals in our previous DBS studies [1-3].

Gender
We will only use male rats, since the oestrogen cycle can interfere with the behavioral outcome and brain biochemistry, such as release of the neurotransmitters dopamine and serotonin [4]. Accounting for sex as a biological variable in all biomedical research is considered fundamental for enhancing rigor and reproducibility in preclinical research. Since we have behavioral and neurochemical outcome measures, we therefore want to reduce the variability in our data by using only male rats.

Number of animals

The estimation on the total number of animals is based on the study described in appendix 1 and our previous experience with DBS and intracranial sampling and recording methods. We estimate to need 14 animals per group for the acute and 20 for the chronic DBS experiment. The acute and chronic DBS experiment will contain groups of rats with DBS or sham for two brain targets.

Power analysis: Literature review and previous experience indicate a significant effect at $\delta=0.3$. Because the primary readout parameters differ between the acute and chronic studies, we estimate the standard deviation for the acute experiment to be $\sigma=0.25$ and for the chronic $\sigma=0.3$. The significance level is $P < 0.05$ and we require a power of $\eta=0.8$. The readout parameter will entail the change in neurotransmitter levels in the hippocampus during acute DBS under general anesthesia and chronic DBS during behavioral testing. The readout parameter for brain activation will entail the change in local field potentials in the hippocampus measured by intracranial electrophysiology recordings and the activity change in specific brain regions measured by PET-CT imaging. We will also use fiber photometry in order to quantify region-specific changes in Calcium influx.

Acute experiment:
$N = 15.7 * (0.25/0.3)^2 = 10.9$

In the acute experiment, we estimate that factors such as complication of surgery and correct electrode locations will lead to 20% of loss per group.

$N = 10.9 / 0.8 = 13.6$ i.e. 14 animals per group.

Chronic experiment:

$N = 15.7 * (0.3/0.3)^2 = 15.7$

In the chronic study, besides factors such as complication of surgery and correct electrode locations we also have to consider possible electrode losses. Therefore, we estimate 25% of loss per group for the chronic study.

$N = 15.7 / 0.75 = 20.9$ i.e. 21 animals per group.

Table 2: Number of animals needed in study 2. DBS, deep brain stimulation; NT, to study neurotransmitter changes; BA, to study changes in brain activity.

Study	Group	Number of animals
2A (Mechanism of action: acute DBS)	NT Fornix DBS	14
	NT Nucleus basalis DBS	14
	NT Fornix sham	14
	NT Nucleus basalis sham	14
	BA Fornix DBS	14
	BA Nucleus basalis DBS	14
	BA Fornix sham	14
	BA Nucleus basalis sham	14
2B (Mechanism of action: chronic DBS)	NT Fornix DBS	21
	NT Nucleus basalis DBS	21
	NT Fornix sham	21
	NT Nucleus basalis sham	21
	BA Fornix DBS	21
	BA Nucleus basalis DBS	21
	BA Fornix sham	21
	BA Nucleus basalis sham	21

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 and secondary aims of this study, respectively, are to identify the changes in neurotransmitters and brain activation induced by acute and chronic DBS. These aims cannot be achieved by use of in vitro experiments, computer modelling or lower animal species because these models do not allow for analysis of neurotransmitters or measuring brain activation in a neural network. The current study can also not be performed in humans because of the following: 1. Studying the neurochemical effects of DBS in healthy humans is not ethical 2. We cannot continuously sample neurotransmitters and or record from different brain regions through intracranial probes in a non-invasive way.

Reduction
The number of animals in this study is based on appendix 1 and experience from previous studies. Only competent and trained persons will carry out the surgical procedure. For the acute experiment we have reduced the number of animals because this is a terminal experiment. We thus do not have to correct for the loss of animals due to the loss of electrode construct. For the chronic experiment we will use the same number of animals as described in appendix 1. Though study 1 and 2 both entail a chronic DBS experiment in memory-impaired rats, we have chosen to not combine these studies. Study 1 will serve to select the optimal stimulation paradigm and identify the effect on neuronal networks. Moreover, the intracranial sampling methods will implement an ipsilateral lesion to the brain tissue, thus making histology and MR imaging impossible in study 1. The studies are therefore carried out separately.

Refinement
By the experience gained in study 1, we will select the behavioral tests and most optimal stimulation parameter to use in the chronic DBS experiment described in section A. Reduction of number of behavioral test will reduce animal discomfort. Similar to study 1, we will adapt the accommodation and the care to the need of the animals. Moreover, only competent and trained persons will carry out the surgical procedure. We will implement humane endpoints.

Explain what measures will be taken to minimise 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. Similarly, if animals show pain during any of the experimental procedures described, they will receive analgesics. To prevent the occurrence of more than predicted animal suffering, we will implement humane endpoints. The persons involved in this experiment possess a strong background of laboratory animal welfare experiences to minimize animal suffering, pain or fear. Furthermore, all materials derived from the experiments involving GMO's will be dealt with appropriately.

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.

N/A

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.

At the first instance we will house all animals in pairs to minimize distress. However, it is possible that rats may damage each other's electrode construct, which in turn could lead to injury, infection, and loss off the electrode construct. Consequently, this can cause a humane endpoint, euthanasia of the animal and a decrease of experimental power. This therefore introduces a consideration of animal welfare (Refinement) versus the Reduction principle of experimental animal ethics. In case only one rat pair damages each other's electrode construct, we will have to house all animals individually. However, to mitigate some of the negative consequences of individual housing, we will use enrichment objects [5].

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.

Local rules of housing, care and treatment will apply.

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 and imaging procedures. Animals will be monitored daily during the experimental procedures stated in section 2A and will be checked for signs of pain or discomfort. Analgesics will be applied to relief suffering e.g. during the pre-, peri- and post-surgical recovery period. Treatment of pain or suffering will be conducted according to the recommendations of GV-SOLAS: Pain management for laboratory animals (May 2015).

I. Other aspects compromising the welfare of the animals

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

Similar to study 1, we will implant an electrode construct on the skull of the animal. The adverse effects and management of these are thus identical as described in appendix 1. However, in this study, we will additionally implant intracranial probes in the brain and inject AAV-GCaMP, which raises the risk for

infection.

Explain why these effects may emerge.

Implanted probes increase the risk for infection.

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

Animals will be visually inspected daily during experiments. Loss of an electrode construct during the postoperative period or behavioral testing and the likelihood of infection is prevented by the extensive experience in behavioral testing and refinement of surgical techniques performed by our research group over the past 10 years. This has recently led to some detailed changes such as bending the DBS cables along the axis of the animal and using sufficiently high cages during behavioral testing. Additionally, completely drying the skull during stereotactic surgery and working aseptic will minimize electrode loss and infections. Stress due to injections will be minimized by skilled injection techniques and handling of the animals. Animals that experience acute adverse effects caused by DBS during behavioral testing (e.g. seizure), will be released from the stimulation immediately (DBS turned off).

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.

Humane endpoints will be based on considerations regarding general health, severe weight loss (20% body weight loss when compared to the pre-study weight), infections, loss of electrode and probe construct and animal behavior considering the model.

Indicate the likely incidence.

We estimate the likely incidence of implementing a humane endpoint at maximally 15%. However, the study of DBS in naïve animals described in appendix 1 is carried out during which we will optimize surgical and animal procedures to reduce this incidence.

K. Classification of severity of procedures

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

25% of the animals will experience non-recovery.
75% of the animals will experience moderate discomfort.

Acute DBS experiment:
- NT: non-recovery
- BA: Stereotactic surgery for virus injection 4 weeks prior to experiment: moderate discomfort due to surgery

Chronic DBS experiment
- Behavioral tests: Mild to moderate discomfort. See table 1 of appendix 1.
- Stereotactic surgery: moderate discomfort due to surgery.
- DBS and EEG measurements: mild discomfort due to new environment.
- Possible individual housing: moderate discomfort due to non-social behavior
- Euthanasia: mild discomfort, due to anesthesia.
- PET imaging: mild discomfort, due to stress of relocation of animals.

Cumulative discomfort for acute and chronic experiments: 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 euthanized 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

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Centrale Commissie Dierproeven

6

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: 3.4.4.3 Type of animal procedure: Long-term DBS in an animal model of disease

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 design of this study is to evaluate deep brain stimulation (DBS) of the fornix or nucleus basalis of Meynert in an animal model of disease. We will evaluate the long-term effects on cognition and (behavioural) side effects. Our findings in study 1 and 2, will guide us in choosing a suitable model of disease for the present study (e.g. animal model, in which brain pathology is accompanied by a decline of a certain neurotransmitter, change in brain activity etc.). This means that the outcomes of study 1 and 2 are essential to predict in which type of dementia DBS can help to alleviate symptoms. Possible choices are transgenic Alzheimer rats or immunotoxin-mediated rat models of dementia (1).

The aim of this study is to evaluate whether DBS is disease-modifying, i.e. whether DBS can have an effect on brain pathology and slow down the progression of memory loss. For this, we will first evaluate cognitive effects and behavioral (side) effects of DBS in a long-term study. The readout parameters for behavioral side effects and the cognitive effect are dependent on the results of the study described in appendix 1. These will entail readout parameters generated by the specific behavioral tests, EEG and fiber photometry recordings that have successfully shown the changes in cognition and behavior in these studies. The readout parameter for the effects on AD brain pathology will entail the anatomic (re)organization of white matter tracts originating from the fornix or nucleus basalis of Meynert and resting activity related brain regions generated by diffusion tensor MRI imaging and resting state functional MRI, respectively. After sacrificing the animals, brains will be thoroughly screened with post-mortem (immuno)histochemistry in order to further evaluate the effects of DBS on brain pathology.

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

Test paradigm	Item	Readout parameter	Discomfort
Barnes maze	memory	number of errors per trial, rate of decline in number of errors and path length	Mild discomfort, results in increased anxiety
Morris water maze	memory	time spent in the platform quadrant, escape latency and swimming speed	Moderate discomfort, animals will be in water
Cued and contextual fear conditioning	memory	percentage of time spent freezing and time to extinction	Moderate discomfort, small electrical shock will be administered
Object location task	memory	ratio of time spent at object in new versus old location	Mild discomfort, results in increased anxiety
Object recognition task	memory	ratio of time spent at new versus old object	Mild discomfort, results in increased anxiety
Y-maze	memory	Ratio left-right discrimination	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
Prepulse inhibition	cognition	startle response	Moderate discomfort, animals will temporarily be in a restrainer
Forced swim task	mood	immobility duration, latency to immobility, swimming duration, climbing duration	Moderate discomfort, animals will be in water
Sucrose intake/preference	mood	volume of sucrose intake corrected for animal weight	Mild discomfort, results in increased anxiety
Food intake	mood	volume of food intake corrected for animal weight	Moderate discomfort, animals will receive restricted feeding regimes or deprivation
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 and locomotion	total distance travelled, average speed and time spent in closed arms	Mild discomfort, results in increased anxiety
Home-cage emergency	impulsivity and anxiety	latency to escape	Mild discomfort, results in increased anxiety

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

This study is a long-term DBS experiment (max. experimental duration of 10 months) and entails DBS, MR imaging, microdialysis, fiber photometry and behavioral tests in freely moving or anaesthetized rats

(as described under point 1.3 Surgery and behavioral experiments). For a summary of the experimental groups see section 2B. The procedures proposed in this study are comparable to those published in literature (2, 3) and previous studies (4-6) in which we used similar imaging methods, DBS and behavioral testing paradigms in animal models for neurodegenerative diseases (either transgenic or immunotoxin-mediated rat models of Alzheimer's).

MRI – baseline
Animals are first subjected to a baseline measurement of neuronal networks using preclinical 7 Tesla MR brain imaging under general anesthesia. An imaging protocol of about 2 hours per animal is applied to obtain high resolution anatomical T2, diffusion tensor imaging, resting state functional MRI and spectroscopy information (7). Subsequently, animals return to their home cage for a minimum of 1 week and, depending on the behavioral paradigm, they are trained for maximally 2 months and then receive brain electrodes.

Surgery and behavioral experiments
DBS electrodes are bilaterally implanted in the fornix or nucleus basalis of Meynert. An EEG electrode is implanted in the hippocampus. Sham animals will undergo the same surgical procedure, but are not stimulated. In the chronic phase, animals will be subjected to behavioral tests for memory, cognition, mood and anxiety. The number and choice of behavioral test as well as stimulation parameters will depend on the outcomes of study 1. Behavioral tests are performed in both DBS on and off conditions and stimulation paradigms (constant, random and EEG responsive stimulation) are applied for maximally 24 hours per day. Next, depending on the outcomes of study 2, we want to measure mechanisms of action in DBS on and off conditions. This can be related to microdialysis, fiber photometry or both. To be able to evaluate the effect of DBS on newly integrated neuronal networks (e.g. hippocampal neurogenesis), that are not detectable by MRI, animals will receive injections with a label that detects proliferative cells (bromodeoxyuridine, maximum 2 times a day for 5 days). To be able to evaluate the effect of DBS on changes in hippocampal theta waves, which cannot be detected by MRI, animals will undergo EEG recordings.

MRI – end
After behavioral testing, the electrode construct is removed under general anesthesia and animals are subjected to the same MRI protocol as performed at baseline. Subsequently, animals are euthanized appropriately for post-mortem analysis of the brain.

Location of MR imaging
Due to the possibility that the MRI scanner may not be available at our university during the study, we will consider to perform this part of the study in a different Dutch or European center with a license to perform animal experiments. Possible locations entail the University of Leuven, Gent, Utrecht or Aachen. In that case we will file a working protocol and / or an ethical application at the local facility. Based on our previous experience, we estimate a maximal experimental time of 10 months per animal.

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

We have based the number of animals on literature review, previous studies performed by our research group and a power analyses. The power analyses is presented in section 2B.

B. The animals

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

Animals
These experiments require male adult rats with AD-like pathology or naïve animals (controls) purchased from a registered breeding facility. Our findings in study 1 and 2, will guide us in choosing a suitable model of disease for the present study (e.g. animal model, in which brain pathology is accompanied by a decline of a certain neurotransmitter, etc.). This means that the outcomes of study 1 and 2 are essential to predict in which type of dementia DBS can help to alleviate symptoms. For this, we will choose an AD

model with the least degree of discomfort, which can answer our research question. Considering animal welfare, it is known that rats with genetically- or chemically-induced AD-like pathology suffer from (progressive) cognitive impairment (8, 9). Contrary to this, basic neurological features such as righting response after being placed on the dorsal side, eye blink, ear twitch, and limb withdrawal in response to tactile stimuli, orienting response to a visual stimuli, startle response following an auditory stimulus, weight and feeding behavior seem to be comparable to age-matched wild-type rats (10, 11). It is difficult to predict the effects on an individual animal, so genetically engineered or immunotoxin-induced animals will be monitored closely to mitigate any unanticipated welfare concerns as they arise.

Gender

We will only use male rats, since the oestrogen cycle can interfere with the behavioral outcome and brain biochemistry, such as release of the neurotransmitters dopamine and serotonin (12). Accounting for sex as a biological variable in all biomedical research is considered fundamental for enhancing rigor and reproducibility in preclinical research. Since we have behavioral and neurochemical outcome measures, we therefore want to reduce the variability in our data by using only male rats.

Number of animals

The estimation on the total number of animals is based on the studies described in appendix 1 and 2 and our previous experience with DBS. This means that results of study 1 and 2 might lead to the exclusion of a DBS group together with its corresponding sham, if it was not able to restore memory loss based on behavioral and physiological parameters. Since we are investigating the potency DBS to reduce side effects and increase the cognitive effect in an animal model of disease, we foresee a larger dropout rate than in study 1 and 2. We therefore estimate to need 22 per group for Alzheimer rats and 16 animals per group for naïve controls (no surgery; we want to investigate whether the memory performance of DBS AD rats is comparable to healthy controls). In total we will have a maximum of 9 groups, DBS and sham stimulation in Alzheimer rats and one control group (see table 2).

Power analysis: Literature review and previous experience indicate a significant effect at $\delta=0.3$ and a standard deviation of $\sigma=0.3$. The significance level is $P < 0.05$ and we require a power of $\eta=0.8$. The readout parameters for behavioral side effects and the cognitive effect are dependent on the results of the study described in appendix 1. These will entail readout parameters generated by the specific behavioral tests, EEG and fiber photometry recordings that have successfully shown the changes in cognition and behavior in these studies. The readout parameter for the effects on AD brain pathology will entail the anatomic (re)organization of white matter tracts originating from the fornix or nucleus basalis of Meynert and resting activity related brain regions generated by diffusion tensor MRI imaging and resting state functional MRI, respectively. After sacrificing the animals, brains will be thoroughly screened with post-mortem (immuno)histochemistry in order to further evaluate the effects of DBS on brain pathology.

$N = 15.7 * (0.3/0.3)^2 = 15.7$

Due to factors such as complication of surgery, correct electrode locations, possible electrode losses and additional dropout due to the disease model, we estimate 35% of loss per group.

$N = 15.7 / 0.65 = 24.2$ e.g. 25 animals per group.

Control animals are naïve rats and will not receive any electrodes, so we do not expect any complication factors.

Table 2: Number of animals needed for study 3. Please note that results of study 1 and 2 might lead to the exclusion of a DBS group together with its corresponding sham, if it was not able to restore memory loss based on behavioral and physiological parameters. Only the most-optimal stimulation parameter derived from study 1 is used here. Control rats are wild-type rats without AD pathology and without DBS. DBS, deep brain stimulation. NT, to study neurotransmitter changes; BA, to study changes in brain activity.

Study	Group	Number of animals
3 (AD-Chronic DBS)	NT AD rat fornix DBS	25
	NT AD rat nucleus basalis DBS	25
	NT AD rat fornix sham	25
	NT AD rat nucleus basalis sham	25
	BA AD rat fornix DBS	25
	BA AD rat nucleus basalis DBS	25
	BA AD rat fornix sham	25
	BA AD rat nucleus basalis sham	25
	control	16

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 and secondary aims of this study, respectively, are to investigate DBS induced cognitive and behavioral side effects in an animal model of disease and to evaluate the underlying mechanism. These aims can only be achieved when using the same experimental procedures as used in appendix 1 and 2, since the methods used in this study are dependent on these studies. These aims cannot be achieved by use of in vitro experiments, computer modelling, lower animal species because these systems do not represent the complete biological system of the brain and do not allow for analysis of complex behavior in conjunction with measuring levels of neurotransmitter or brain activation. The current study can also not be performed in humans because of the following: 1. Studying the effects of different drug treatments on behavior and changes in neurotransmitters and brain activation in humans is not ethical, and 2. We cannot continuously sample neurotransmitters and or record from different brain regions through intracranial probes in a non-invasive way.

Reduction

The number of animals in this study is based on appendix 1 and 2. The intermediate results of these studies could reduce group size if possible. Study 1 will provide insights to the most optimal stimulation parameter to restore memory loss. Study 2 will provide insights which mechanism of action is responsible for restoring memory loss. In study 3 we will therefore only proceed with most optimal DBS target of study 1 and the intracranial sampling producing significant effects in study 2, which might reduce the number of animals. Moreover, only competent and trained persons will carry out the surgical procedure.

Refinement

We will choose the most suitable animal model for Alzheimer's for our study to reduce experimental duration time and we will further refine the model and stimulation paradigms for usage in future experiments. If more models are suitable to answer our research question, we will choose the one with the least degree of discomfort. By the experience gained in study 2, we can select the most suitable test to evaluate mechanisms of action of DBS (measure levels of neurotransmitters or measure brain

activation or both). Animal procedures that could not define a difference between groups will not be selected to use in this study. We will adapt the care to the need of the animals at the different stages of the experiment (e.g. recovery-food during recovery period after surgery, cage enrichment in case animals have to be single-housed, etc). Moreover, only competent and trained persons will carry out the surgical procedure.

Explain what measures will be taken to minimise 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. Similarly, if animals show pain during any of the experimental procedures described, they will receive analgesics. To prevent the occurrence of more than predicted animal suffering, we will implement humane endpoints. 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.

N/A

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.

At the first instance we will house all animals in pairs to minimize distress. However, it is possible that rats may damage each other's electrode construct, which in turn could lead to injury, infection, and loss off the electrode construct. Consequently, this can cause a humane endpoint, euthanasia of the animal and a decrease of experimental power. This therefore introduces a consideration of animal welfare (Refinement) versus the Reduction principle of experimental animal ethics. In case only *one* rat pair damages each other's electrode construct, we will have to house *all* animals individually. However, to mitigate some of the negative consequences of individual housing, we will use enrichment objects (13).

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 and imaging procedures. Animals will be monitored daily during the experimental procedures stated in section 2A and will be checked for signs of pain or discomfort. Analgesics will be applied to relief suffering e.g. during the pre-, peri- and post-surgical recovery period. Treatment of pain or suffering will be conducted according to the recommendations of GV-SOLAS: Pain management for laboratory animals (May 2015).

I. Other aspects compromising the welfare of the animals

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

Animals with AD-like brain pathology could show potential DBS-related side effects different from the ones of naïve rats. These side-effects might lead to an adverse reaction and increase in discomfort or increased anxiety. Other possible adverse effects and measures to minimize severity of adverse effects on the animal's welfare are identical to the studies described in appendix 1 and 2.

Explain why these effects may emerge.

A transgenic model refers to animals in which the genome is altered through the use of genetic engineering techniques, while in an immunotoxin-mediated model we will inject an immunotoxin into the brain to initiate memory loss and mimic AD-like pathology. Depending on the AD model we will use, adverse effects might be related to brain pathology or the administered immunotoxins. Nevertheless, we will choose an AD model with the least degree of discomfort, which can answer our research question. It is known that rats with genetically- or chemically-induced AD-like pathology suffer from (progressive) cognitive impairment (8, 9). Contrary to this, basic neurological features such as righting response after being placed on the dorsal side, eye blink, ear twitch, and limb withdrawal in response to tactile stimuli, orienting response to a visual stimuli, startle response following an auditory stimulus, weight and feeding behavior seem to be comparable to age-matched wild-type rats (10, 11). It is difficult to predict the effects on an individual animal, so genetically engineered or immunotoxin-induced animals will be monitored closely to mitigate any unanticipated welfare concerns as they arise.

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

Animals will be visually inspected daily during experiments. Loss of an electrode construct during the postoperative period or behavioral testing is prevented by the extensive experience in behavioral testing and refinement of surgical techniques performed by our research group over the past 10 years. This has recently led to some detailed changes such as bending the DBS cables along the axis of the animal and using sufficiently high cages during behavioral testing. Additionally, completely drying the skull during stereotactic surgery and working aseptic will minimize electrode loss. Stress due to injections will be minimized by skilled injection techniques and handling of the animals. For animals that experience severe adverse effects caused by the nature of the AD model, humane endpoints will be applied.

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.

Humane endpoints will be based on considerations regarding general health, severe weight loss (20% body weight loss when compared to the pre-study weight), infections, loss of electrode construct and animal behavior considering the model. Besides cognitive decline, various AD or immunotoxin models do not show evidence for other neurological or physiological symptoms (14). If any unexpected clinical signs compromising animal welfare will be detected, we will implement humane endpoints.

Indicate the likely incidence.

We estimate the incidence of implementing a humane endpoint at maximally 25%. In the course of this research proposal we will optimize surgical and animal procedures to reduce this incidence.

K. Classification of severity of procedures

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

-Behavioral tests: Mild to moderate discomfort. See table 1.
-Stereotactic surgery: moderate discomfort due to surgery.
-DBS and EEG measurements: mild discomfort due to new environment.
-Drug treatment: mild discomfort due to repeated injections
-Alzheimer animal model: moderate discomfort due to neurodegeneration.
-Possible individual housing: moderate discomfort due to non-social behavior
-PET-CT: mild discomfort, due to anesthesia and relocation of animals.
-Euthanasia: mild discomfort, due to anesthesia.

Cumulative discomfort: 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 euthanized 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

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Centrale Commissie Dierproeven

7

Appendix
Description animal procedures

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

1

General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	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: 3.4.4.4 Type of animal procedure: The role of neuropharmacology in DBS for dementia-related disorders

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 design of this study is an evaluation of a combined intervention of drug treatments and deep brain stimulation (DBS) of the fornix or nucleus basalis in an animal model of disease. We will evaluate the effect on (behavioral) side effects and additional cognitive effect when DBS is combined with a drug treatment. We will use the same model as in appendix 3. Our findings in study 1 and 2, will guide us in choosing a suitable model of disease for the present study (e.g. animal model, in which brain pathology is accompanied by a decline of a certain neurotransmitter, change in brain activity etc.) This means that the outcomes of study 1 and 2 are essential to predict in which type of dementia DBS can help to alleviate symptoms. Possible choices are transgenic Alzheimer rats or immunotoxin-mediated rat models of dementia [1].

The primary aim of this study is to identify a drug that reduces DBS induced behavioral side effects and/or increases the cognitive effect of DBS. The readout parameters for behavioral side effects and the cognitive effect are dependent on the results of the study described in appendix 1. These will entail readout parameters generated by the specific behavioral tests and EEG recordings that have successfully shown the changes in cognition and behavior in these studies.

The secondary aim of this study is to evaluate the underlying mechanism of the combined intervention in reducing behavioral side effects and increasing cognitive effects. The readout parameters are dependent on the results of the study described in appendix 2. These will entail the readout parameters generated by the intracranial sampling and recordings methods and imaging methods that have

successfully shown changes in neurotransmitters and/or brain activation in study 2.

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

Test paradigm	Item	Readout parameter	Discomfort
Barnes maze	memory	number of errors per trial, rate of decline in number of errors and path length	Mild discomfort, results in increased anxiety
Morris water maze	memory	time spent in the platform quadrant, escape latency and swimming speed	Moderate discomfort, animals will be in water
Cued and contextual fear conditioning	memory	percentage of time spent freezing and time to extinction	Moderate discomfort, small electrical shock will be administered
Object location task	memory	ratio of time spent at object in new versus old location	Mild discomfort, results in increased anxiety
Object recognition task	memory	ratio of time spent at new versus old object	Mild discomfort, results in increased anxiety
Y-maze	memory	Ratio left-right discrimination	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
Prepulse inhibition	cognition	startle response	Moderate discomfort, animals will temporarily be in a restrainer
Forced swim task	mood	immobility duration, latency to immobility, swimming duration, climbing duration	Moderate discomfort, animals will be in water
Sucrose intake/preference	mood	volume of sucrose intake corrected for animal weight	Mild discomfort, results in increased anxiety
Food intake	mood	volume of food intake corrected for animal weight	Moderate discomfort, animals will receive restricted feeding regimes or deprivation
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 and locomotion	total distance travelled, average speed and time spent in closed arms	Mild discomfort, results in increased anxiety
Home-cage emergency	impulsivity and anxiety	latency to escape	Mild discomfort, results in increased anxiety

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

This study entails a combined intervention of drug treatments and chronic DBS (maximal experimental duration of 10 months), EEG recordings, behavioral tests, and intracranial sampling and recording methods, MRI/PET-CT imaging and labelling of proliferative cells (e.g. bromodeoxyuridine) in animal models for neurodegenerative diseases (either transgenic or immunotoxin-mediated rat models of Alzheimer's). The definite animal procedures that will be performed in this study are dependent on the results of the studies described in appendix 1, 2 and 3.

Drug treatments
We estimate to use two different drug treatments to enhance DBS and/or reduce side effects. The choice for these drug treatments is dependent on the underlying mechanism of DBS and generation of side effects that is exposed by studies 1 and 2. However we predict it will be modulators of the glutamatergic and cholinergic neurotransmitter system such as memantine, an NMDA receptor antagonist and rivastigmine, an acetylcholinesterase inhibitor. Depending on the drug treatment to use, administration will either be intracranial, intraperitoneal, subcutaneous or oral. Different doses will be administered to see if there is a dose-response curve and to see which dose is most optimal for the combination therapy with DBS. We have also included a vehicle-control group (see Table 2).

Animal procedures
To evaluate the efficacy of DBS and drug treatments we will use the same behavioral tests and intracranial sampling, recordings, imaging and labelling methods that have displayed the underlying mechanism of DBS in studies 1, 2 and 3. Animal procedures in this study are thus similar to the procedures used in studies 1, 2 and 3. Stereotactic surgery will be performed as stated in appendix 2. Intracranial, intraperitoneal or subcutaneous injections of the different drug treatments, dependent on the nature of the drug are ultimately chosen. The outcomes of these tests will allow us to assess whether a drug is effective in enhancing the cognitive effect or reducing behavioral side effects of DBS, but also what the impact of this treatment is on the levels of neurotransmitters and activation of brain regions. The expected minimum number of behavioral tests is 4 and the maximum number of behavioral tests is 7. During treatment, by intravenous punctures and intracranial probes, blood (according to NC3R guidelines blood sampling decision tree) and CFS will be collected on a maximum weekly basis to monitor levels of inflammatory proteins or other markers specific for the possible side effects of the used drug treatments. After the proposed animal procedures, all rats are euthanized as appropriate and the brain is obtained for further histological processing. Since this study entails a chronic DBS experiment similar to appendix 1, 2 and 3, we estimate a maximal experimental duration time of 10 months per animal.

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

We have based the number of animals on literature review, previous studies performed by our research group and a power analyses. The power analyses is presented in section 2B.

B. The animals

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

Animals
These experiments require male adult rats with AD-like pathology or naïve animals (controls) purchased from a registered breeding facility. Our findings in study 1 and 2, will guide us in choosing a suitable model of disease for the present study (e.g. animal model, in which brain pathology is accompanied by a decline of a certain neurotransmitter, etc.) This means that the outcomes of study 1 and 2 are essential to predict in which type of dementia DBS can help to alleviate symptoms. For this, we will choose an AD model with the least degree of discomfort, which can answer our research question. Considering animal welfare, it is known that rats with genetically- or chemically-induced AD-like pathology suffer from (progressive) cognitive impairment [2, 3]. Contrary to this, basic neurological features such as righting response after being placed on the dorsal side, eye blink, ear twitch, and limb withdrawal in response to tactile stimuli, orienting response to a visual stimuli, startle response following an auditory stimulus, weight and feeding behavior seem to be comparable to age-matched wild-type rats [4, 5]. It is difficult to predict the effects on an individual animal, so genetically engineered or immunotoxin-induced animals will be monitored closely to mitigate any unanticipated welfare concerns as they arise.

Gender
We will only use male rats, since the oestrogen cycle can interfere with the behavioral outcome and brain biochemistry, such as release of the neurotransmitters dopamine and serotonin [6]. Accounting for sex as a biological variable in all biomedical research is considered fundamental for enhancing rigor and reproducibility in preclinical research. Since we have behavioral and neurochemical outcome measures, we therefore want to reduce the variability in our data by using only male rats.

Number of animals
The estimation on the total number of animals is based on the studies described in appendix 1 and 2 and our previous experience with DBS. This means that results of study 1 and 2 might lead to the exclusion of a DBS group together with its corresponding sham, if it was not able to restore memory loss based on behavioral and physiological parameters. Since we are investigating the potency of a drug treatment in combination with DBS to reduce side effects and increase the cognitive effect in an animal model of disease, we foresee a larger dropout rate than in study 1 and 2. We therefore estimate to need 22 per group for Alzheimer rats and 16 animals per group for naïve controls (no surgery). In total we will have a maximum of 9 groups, DBS and sham stimulation in Alzheimer rats and one control group (see table 2).

Power analysis: Literature review and previous experience indicate a significant effect at $\delta=0.3$ and a standard deviation of $\sigma=0.3$. The significance level is $P < 0.05$ and we require a power of $\eta=0.8$. The readout parameters for behavioral side effects and the cognitive effect are dependent on the results of the study described in appendix 1. These will entail readout parameters generated by the specific behavioral tests and EEG recordings that have successfully shown the changes in cognition and behavior in these studies. The readout parameters to evaluate the underlying mechanism of the combined intervention are dependent on the results of the study described in appendix 2. These will entail the readout parameters generated by the intracranial sampling and recordings methods and imaging methods that have successfully shown changes in neurotransmitters and/or brain activation in study 2.

$N = 15.7 * (0.3/0.3)^2 = 15.7$

Due to factors such as complication of surgery, correct electrode locations, possible electrode losses and additional dropout due to the disease model, we estimate 35% of loss per group.

$N = 15.7 / 0.65 = 24.2$ e.g. 25 animals per group.

Control animals are naïve rats and will not receive any electrodes, so we do not expect any complication factors.

Table 2: Number of animals needed for study 4. Please note that results of study 1 and 2 might lead to the exclusion of a DBS group together with its corresponding sham, if it was not able to restore memory loss based on behavioral and physiological parameters. Control rats are wild-type rats without AD pathology and DBS, but with the injection of vehicle. DBS, deep brain stimulation. NT, to study neurotransmitter changes; BA, to study changes in brain activity.

Study	Group	Number of animals
4 (AD-Chronic DBS + drugs)	NT AD rat fornix DBS	25
	NT AD rat nucleus basalis DBS	25
	NT AD rat fornix sham	25
	NT AD rat nucleus basalis sham	25
	BA AD rat fornix DBS	25
	BA AD rat nucleus basalis DBS	25

	BA AD rat fornix sham BA AD rat nucleus basalis sham control	25 25 16
4 (AD-Chronic DBS + vehicle)	NT AD rat fornix DBS NT AD rat nucleus basalis DBS NT AD rat fornix sham NT AD rat nucleus basalis sham BA AD rat fornix DBS BA AD rat nucleus basalis DBS BA AD rat fornix sham BA AD rat nucleus basalis sham	25 25 25 25 25 25 25 25

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 and secondary aims of this study, respectively, are to identify a drug treatment that reduces DBS induced behavioral side effects and/or increases the cognitive effect of DBS in an animal model of disease and to evaluate the underlying mechanism. These aims can only be achieved when using the same experimental procedures as used in appendix 1 and 2, since the methods used in this study are dependent on these studies. These aims cannot be achieved by use of in vitro experiments, computer modelling, lower animal species because these systems do not represent the complete biological system of the brain and do not allow for analysis of complex behavior in conjunction with measuring levels of neurotransmitter or brain activation. Also, we will choose a model chosen, which is the best representative for the Alzheimer patient population, that is receiving DBS therapy. The current study can also not be performed in humans because of the following: 1. Studying the effects of different drug treatments on behavior and changes in neurotransmitters and brain activation in humans is not ethical, and 2. We cannot continuously sample neurotransmitters and or record from different brain regions through intracranial probes in a non-invasive way.

Reduction

The number of animals in this study is based on appendix 1 and 2. The intermediate results of these studies could reduce group size if possible (i.e. proceed only with the most optimal DBS target of study 1 and intracranial sampling method producing significant effects in study 2). Moreover, only competent and trained researchers will carry out the surgical procedure.

Refinement

We will choose the best animal model for Alzheimer's to reduce experimental duration time and we will only use the most optimal stimulation paradigm derived from study 1. By the experience gained in study 2, we can better select the animal procedure to measure levels of neurotransmitters or measure brain activation. Animal procedures that could not define a difference between groups will not be selected to use in this study. Likewise to study 1 and 2, we will adapt the accommodation and care to the need of the animals. Moreover, only competent and trained researchers will carry out the surgical procedure.

Explain what measures will be taken to minimise 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. Similarly, if animals show pain during any of the experimental procedures described, they will receive analgesics. To prevent the occurrence of more than predicted animal suffering, we will implement humane endpoints. 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.

N/A

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.

At the first instance we will house all animals in pairs to minimize distress. However, it is possible that rats may damage each other's electrode construct, which in turn could lead to injury, infection, and loss off the electrode construct. Consequently, this can cause a humane endpoint, euthanasia of the animal and a decrease of experimental power. This therefore introduces a consideration of animal welfare (Refinement) versus the Reduction principle of experimental animal ethics. In case only *one* rat pair damages each other's electrode construct, we will have to house *all* animals individually. However, to mitigate some of the negative consequences of individual housing, we will use enrichment objects [7].

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 and imaging procedures. Animals will be monitored daily during the experimental procedures stated in section 2A and will be checked for signs of pain or discomfort. Analgesics will be applied to relief suffering e.g. during the pre-, peri- and post-surgical recovery period. Treatment of pain or suffering will be conducted according to the recommendations of GV-SOLAS: Pain management for laboratory animals (May 2015).

I. Other aspects compromising the welfare of the animals

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

Animals could show a potential side effect which might lead to an adverse reaction and increase in discomfort or increased anxiety. Other possible adverse effects and measures to minimize severity of adverse effects on the animal's welfare are identical to the studies described in appendix 1 and 2.

Explain why these effects may emerge.

The drugs could cause side effects that result in animal discomfort. Because the selection of drugs is dependent on study 2, we cannot predict the side effects to expect. However, because we expect the drugs to be modulators of the glutamatergic and/or cholinergic neurotransmitter system, we can expect side effects such as increase in locomotor activity, food and water consumption. From the AD animal model it is known that rats with genetically- or chemically-induced AD-like pathology suffer from

(progressive) cognitive impairment [2, 3]. Contrary to this, basic neurological features such as righting response after being placed on the dorsal side, eye blink, ear twitch, and limb withdrawal in response to tactile stimuli, orienting response to a visual stimuli, startle response following an auditory stimulus, weight and feeding behavior seem to be comparable to age-matched wild-type rats [4, 5]. It is difficult to predict the effects on an individual animal, so genetically engineered or immunotoxin-induced animals will be monitored closely to mitigate any unanticipated welfare concerns as they arise.

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

Animals will be visually inspected daily during experiments. Loss of an electrode construct during the postoperative period or behavioral testing is prevented by the extensive experience in behavioral testing and refinement of surgical techniques performed by our research group over the past 10 years. This has recently led to some detailed changes such as bending the DBS cables along the axis of the animal and using sufficiently high cages during behavioral testing. Additionally, completely drying the skull during stereotactic surgery and working aseptic will minimize electrode loss. Stress due to injections will be minimized by skilled injection techniques and handling of the animals. For animals that experience severe adverse effects caused by the nature of the AD model or the drug treatment, humane endpoints will be implemented.

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.

Humane endpoints will be based on considerations regarding general health, severe weight loss (20% body weight loss when compared to the pre-study weight), infections, loss of electrode construct and animal behavior considering the model. Besides cognitive decline, various AD or immunotoxin models do not show evidence for other neurological or physiological symptoms [8]. If any unexpected clinical signs compromising animal welfare will be detected, we will implement humane endpoints.

Indicate the likely incidence.

We estimate the likely incidence of implementing a humane endpoint at *maximally* 25%. In the course of this research proposal we will optimize surgical and animal procedures to reduce this incidence.

K. Classification of severity of procedures

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

-Behavioral tests: Mild to moderate discomfort. See table 1.
-Stereotactic surgery: moderate discomfort due to surgery.
-DBS and EEG measurements: mild discomfort due to new environment.
-Drug treatment: mild discomfort due to repeated injections, mild discomfort due to possible side effects
-Alzheimer animal model: moderate discomfort due to neurodegeneration.
-Possible individual housing: moderate discomfort due to non-social behavior
-PET-CT: mild discomfort, due to anesthesia and relocation of animals.
-Euthanasia: mild discomfort, due to anesthesia.

Cumulative discomfort: 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 euthanized 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

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7. Van Loo PL, Van de Weerd HA, Van Zutphen LF, Baumans V. Preference for social contact versus environmental enrichment in male laboratory mice. Laboratory animals. 2004;38(2):178-88.
8. Do Carmo S, Cuello AC. Modeling Alzheimer's disease in transgenic rats. Molecular Neurodegeneration. 2013;8(1):37.

Van: [redacted]
Verzonden: maandag 13 februari 2017 19:46
Aan: Info-zbo
CC: [redacted]
Onderwerp: AW: Aanvullende informatie AVD107002016785

8

Categorieën: Dossier: [redacted]

Dear [redacted]
we thank the committee for their valid concern. After DBS surgery the animals will recover for 1-2 weeks before behavioral testing begins. Only at the end of behavioral testing we will perform MRI. In the unlikely event that the MRI scanner is not available at Maastricht University, we will transport the animals to a different facility. The time between DBS surgery and transport will thus be around 12 weeks or more. Animals will be under general anesthesia during surgical procedures and will receive appropriate analgesics during the post-operative recovery period. If animals show pain during any of the experimental procedures described, they will first receive analgesics. We will monitor the animals daily and implent humane endpoints on considerations regarding general health, severe weight loss (20% body weight loss), infections, loss of electrode construct and animal behavior throughout the study. We will thus transport only healthy animals.

With kind regards,
[redacted]

[redacted] | Maastricht University | The Netherlands
[redacted]

Von: Info-zbo
Gesendet: Montag, 13. Februar 2017 16:38
An: [redacted]
Betreff: RE: Aanvullende informatie AVD107002016785

Geachte [redacted]
Wij hebben uw antwoord ontvangen. De CCD vindt het bezwaarlijk dat dieren na het ondergaan van een operatie naar een andere instelling vervoerd worden. Kunt u aangeven hoe lang de dieren de tijd krijgen om te herstellen van de operatie voordat ze vervoerd zullen worden en op welke wijze geborgd wordt dat de dieren voldoende hersteld zijn van de operatie om vervoerd te kunnen worden?

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.
Met vriendelijke groet,

[redacted]
Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl

.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl (Let op: nieuw e-mail adres)

Van: H[redacted]
Verzonden: vrijdag 3 februari 2017 13:11
Aan: info@zbo-ccd.nl

CC: [redacted]
Onderwerp: WG: Aanvullende informatie AVD107002016785
Dear Sir or Madam,
please find our response regarding your question on application AVD107002016785 below:
In the unlikely event that the MRI scanner is not available at Maastricht University, we mentioned in the proposal that we consider scanning the animals at a different location. For this we want to transport the animals between the locations and will file a working protocol and / or an ethical application at the local facility. Unfortunately, it is not possible to perform the entire experiment at an external location, because the DBS and EEG equipment is managed and shared in the lab environment of Maastricht University. Transportation of animals will take place with care and a dedicated animal transport service to cause the least amount of discomfort. Animals will also be able to recover for 5 days after transportation.
We hope this information meets your satisfaction.
With kind regards,
[redacted]

From: Info-zbo [mailto:info@zbo-ccd.nl]
Sent: donderdag 26 januari 2017 14:39
To: [redacted]
Cc: [redacted]
Subject: Aanvullende informatie AVD107002016785
Geachte [redacted]
Op 15 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project 'Deep brain stimulation to restore memory loss' met aanvraagnummer AVD107002016785. Wij hebben nog een vraag over uw aanvraag.
U geeft aan te overwegen, bij het niet beschikbaar zijn van de MRI scanner in de eigen instelling, uit te wijken naar een andere locatie. Uit uw aanvraag blijkt echter niet of in dergelijke situaties dieren die al in een proef zijn opgenomen tussen beide locaties vervoerd zullen worden of dat in die situaties de gehele proef op de andere locatie uitgevoerd zal worden. U wordt verzocht dit te verhelderen.
Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP.
Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.
Met vriendelijke groet,

[redacted]
Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**

.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl (Let op: nieuw e-mail adres)

9

DEC-advies PV 2016-014/[redacted]

Preambule;
De DEC-UM verzoekt U eventuele aanvullende vragen rechtstreeks aan de aanvrager te stellen met een afschrift aan de DEC-UM.

A. Algemene gegevens over de procedure

1. **Aanvraagnummer:** 10700

2. **Titel van het project:** *Deep brain stimulation to restore memory loss?*

3. **Titel van de NTS:** *Diepe hersenstimulatie bij geheugenverlies.*

4. **Type aanvraag:**
   X **nieuwe** aanvraag projectvergunning

5. **Contactgegevens DEC:**
   - naam DEC; *DEC-UM*
   - telefoonnummer contactpersoon; [redacted]
   - e-mailadres contactpersoon; [redacted]

6. **Adviestraject:** (data dd-mm-jjjj):
   X ontvangen door DEC-UM 03-11-2016
   ☐ aanvraag compleet
   X in vergadering besproken 11-11-2016
   ☐ anderszins behandeld
   ☐ termijnonderbreking(en) van / tot
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☐ aanpassing aanvraag
   ☐ advies aan CCD

7. **Afstemming IvD:**
   - De aanvrager heeft het projectvoorstel afgestemd met de IvD *dd. 03-11-2016*.

8. **Eventueel horen van aanvrager**: ***N.V.T***.

9. **Correspondentie met de aanvrager:**
   - Datum 16-11-2016
   - Gestelde vragen en antwoorden:

3 Algemene projectbeschrijving
3.1 Achtergrond
Algemene opmerkingen:

1. **Opmerking:** Het verdient aanbeveling de noodzaak tot het gebruik van mannelijke dieren (nog) beter te onderbouwen.
   Reactie:
   We will only use male rats, since the estrogen cycle can both interfere with behavior, but also neurochemistry. Accounting for sex as a biological variable in all biomedical research is considered fundamental for enhancing rigor and reproducibility in preclinical research.

Since we have behavioral and neurochemical outcome measures, we therefore want to reduce the variability in our data by using only male rats. We have now added this information to the different appendices.

2. **Opmerking:** Gelieve de uitleesparameters van de poweranalyses te vermelden op de daartoe strekkende plaats in de bijlagen.
   Reactie:
   We thank the reviewers for this suggestion and have added the outcome measures of the power analyses to the different appendices.

3. **Opmerking:** Gelieve onder de humane eindpunten aan te geven over welke periode een gewichtsverlies van 25% relevant wordt.
   Reactie:
   We will employ a humane endpoint if the animal loses 25% of body weight when compared to his pre-study weight. We have now added this information to the different appendices.

Vraag:
1. Er wordt gesproken over een combinatie van DBS en medicatie. Valt het hierbij niet aan te bevelen DBS (en eventuele combinatie met nieuwe medicamenten) ook te vergelijken met de standaard medicatie voor AD?
   Antwoord:
   Standard medication for AD are acetylcholinesterase inhibitors (donezepil, galantamine, rivastigmine) and NMDA receptor antagonists (memantine). These medications therefore have an impact on the cholinergic and glutamatergic system. In fact, for the current project proposal we would like to make use of the standard treatment modalities and try to combine them with DBS to enhance the therapeutic effects. We have stated this now more clearly in the project proposal.

3.3. Belang
Vraag:
1. Kunt U iets specifieker zijn over de bevindingen van de phase-I klinische trial? Is Uw studieopzet (b.v. instellingen DBS) hierop aangepast?
   Antwoord:
   Laxton et al. performed the first DBS study in which six patients with mild AD were implanted with electrodes in the vicinity of the fornix. After an intraoperative evaluation of stimulation to survey for recollective experiences and adverse effects, patients received chronic high frequency DBS for a period of 12 months (3.0–3.5 V, 130 Hz and 90 µs pulse width). The authors have found that the application of DBS in the hypothalamus/fornix vicinity was safe and triggered neural activity in the memory circuit, including the entorhinal and hippocampal areas. PET scans showed an early and striking reversal of the impaired glucose utilization in the temporal and parietal lobes that was maintained after 12 months of continuous stimulation. Evaluation of the Alzheimer's Disease Assessment Scale cognitive subscale and the Mini Mental State Examination suggested possible improvements and slowing the progression of memory loss at 6 and 12 months, especially in patients that were less severely affected at the time of surgery. In fact, 2 out of 6 patiens showed cognitive improvements, 1 patient remained stable and the other 3 deteriorated. Indeed, our presented project proposal is partly build upon this study. Because the clinical results were inconclusive, we feel that it is important to investigate the effects of DBS on the fornix with a variety of stimulation parameters and also to examine neurochemical and neurophysiological responses.
   With the studies proposed here, we would like to provide robust scientific evidence for the application of DBS in memory-related disorders.
   We also would like to test, whether fornix DBS or nucleus basalis of Meynert DBS (another DBS target, which has been investigated in the clinical setting) is more favorable.

There is a foreseeable possibility that findings of this proposal will facilitate the investigation and management of DBS in patients with memory impairment. We have now described the findings of the phase I trial in more detail and mentioned how our proposed experiments will contribute to the application of DBS in memory-related disorders.

3.4 Onderzoeksstrategie

3.4.1

1. **Vraag:** Het gros van alle gedragstesten leent zich niet voor herhaaldelijk gebruik. Hoe vangt U dit op gezien Uw plan tijdens zowel on als off stimulatie te testen binnen hetzelfde dier?
   Antwoord:
   The reviewers are correct, we need to re-phrase our sentence in the project proposal. In fact, we have sham groups included in all the appendices. Only DBS groups receive "on-stimulation". Sham groups have electrodes attached to their constructs, but the stimulator is switched off ("off-stimulation").We have now stated this more clearly under 3.4.1 of the project proposal.

2. **Vraag:** Kan men subacute en chronisch effecten uitsluiten wanneer men off-stimulatie na on-stimulatie test?
   Antwoord:
   Please see the response to question 1 above (3.4.1-1).

3.4.2

1. **Vraag:** Wat betreft celproliferatie, kan men subacute en chronisch effecten uitsluiten wanneer men off-stimulatie na on-stimulatie test?
   Antwoord:
   Please see the response to question 1 above (3.4.1-1).

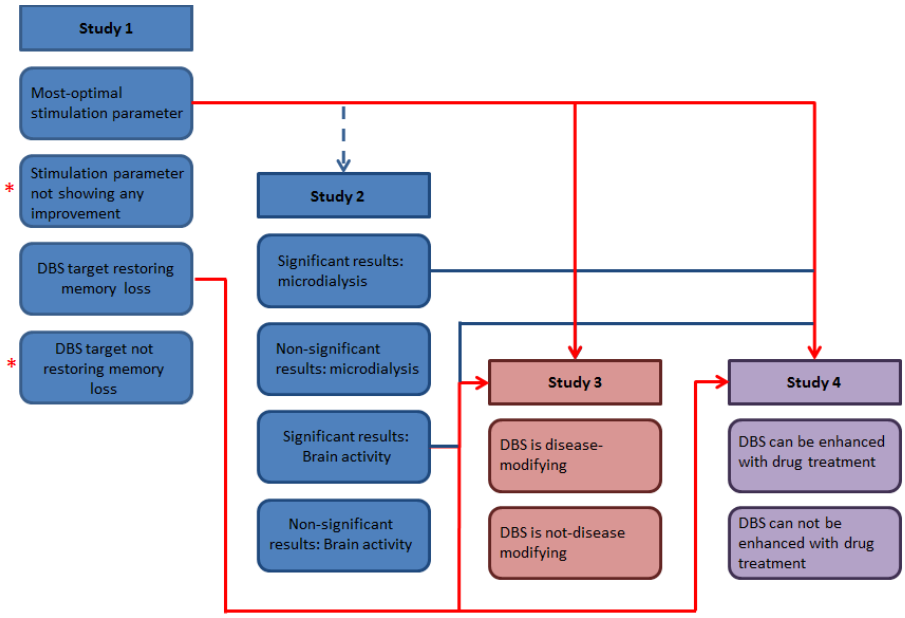
2. **Vraag:** De go-no go opzet is in deze studie wellicht te voorzichtig, daar de aard van het geheugenverlies bij Study 1 (en effecten van DBS daarbij) niet gelijk hoeven te zijn aan de aard van het geheugenverlies bij AD ratmodellen. Het gebrek aan een effect bij Study 1 lijkt zo niet direct van invloed op de kans op een effect bij de volgende studies. Valt het niet aan te bevelen de optimalisatie zoals bij Study 1 voorgesteld direct binnen een AD rattenmodel te doen?
   Antwoord:
   The reviewers raise a valid concern. In the proposed project, however, both study 1 and study 2 are needed in order to guide us in choosing a suitable model of disease for the present study (e.g. animal model, in which brain pathology is accompanied by a decline of a certain neurotransmitter, change in brain activity etc.). This means that the outcomes of study 1 and 2 are essential to predict in which type of dementia DBS can help to alleviate symptoms. Until now, DBS for dementia-related disorders has been inconclusive in the clinical setting. This is mainly, because little is known about mechanisms of action or optimal protocols. One of the main conclusions of a recent phase II trial was that the stimulation parameters applied to AD patients are not disease-specific (Lozano et. al, J Alz Dis, 2016). Evidently, a major drawback of the current neuromodulation approaches is that the clinical application of DBS is moving faster than the scientific evidence supporting or discouraging its application. With study 1 and study 2, we will therefore optimize stimulation protocols for different target areas and define potential mechanisms of action and apply this information to study 3 and 4. Moreover, our suggested scopolamine-induced rat model of dementia is easier to handle and induces acute memory loss, which is more relevant to answer our research question (appendix 1).

   If we would investigate optimal stimulation parameters in a randomly chosen animal model of dementia (e.g. transgenic AD rat), we will not be able to answer our research question in a straightforward fashion.

In most AD models, memory loss is usually progressive/does not have an acute onset and therefore comparing different stimulation parameters will be more laborious.

3. **Vraag:** Kan men subacute en chronisch effecten uitsluiten wanneer men achtereenvolgens verschillende stimulatieparameters varieert (low-high frequency; meerdere amplitudes; meerdere pulse-widths)?
   Antwoord:
   We will always apply a minimum of 24h stimulation-off period between different stimulation parameters and have added this information to the project proposal. We have performed a similar paradigm before (Hescham et al., Brain Stim, 2013) and believe that a short DBS stimulation period during behavioral testing (i.e. under 5 min) is insufficient to induce pronounced long-term changes.

3.4.3
Algemene opmerking:
1. Figuur: wellicht optimale stimulatieparameter en hersengebied samennemen en de meest veelbelovende combinatie testen in Study 3&4?
   Reactie:
   We thank the reviewers for their suggestion. In fact, we intended to do so (test the most optimal stimulation parameter and DBS target in study 3&4). Below, we have highlighted the corresponding go-conditions in red.



Algemene vragen/opmerkingen appendices:

- Bij alle vier appendices is de geschatte uitval in B gelijk aan het percentage dieren waarbij naar schatting van de onderzoekers een humaan eindpunt van toepassing is, in J. Er zijn toch meer redenen voor uitval dan het bereiken van humane eindpunten? Zo ja, dan dient het percentage drop-outs hoger te zijn dan dat bij humane eindpunten.
  Antwoord:
  *The reviewers are correct. We estimated the incidence of implementing a humane endpoint at **maximally** 25%. With this number, we wanted to include all possible scenarios of the predicted drop-out rate mentioned under B.*
  However, there needs to be a clear distinction, between implementing a humane endpoint and expected drop-out rates.
  Implementing a humane endpoint results in euthanasia of animals which are close to suffering and the judgement of euthanasia is based on predefined clinical signs.

Loss of extra animals can be due to unforeseen matters like electrode loss, intraoperative death, etc. Also, we can only verify correct electrode placement post-mortem, which poses another potential drop-out factor. Because of the abovementioned reasons, we should decrease our estimation of implementing a humane endpoint and have therefore adapted the numbers in the revised appendices.

- Dat 35% van de dieren ten gevolge van een humaan eindpunt uitvalt, vindt de DEC-UM erg hoog. (appendix 3 en 4, laatste zin onderdeel J). Is het niet aan te bevelen de experimentele handelingen vooraf beter te optimaliseren?
  Antwoord:
  *Our response to the previous question is also relevant here. Because of the AD rat model (either transgenic or neurotoxin-induced) we estimated the incidence of implementing a humane endpoint higher than for the first two appendices (**maximally** 35%). With this number, we wanted to include all possible scenarios of the predicted drop-out rate mentioned under B. However, there needs to be a clear distinction, between implementing a humane endpoint and expected drop-out rates. Implementing a humane endpoint results in euthanasia of animals which are close to suffering and the judgement of euthanasia is based on predefined clinical signs. Loss of extra animals can be due to unforeseen matters like electrode loss, intraoperative death, etc. Also, we can only verify correct electrode placement post-mortem, which poses another potential drop-out factor. Because of the abovementioned reasons, we should decrease our estimation of implementing a humane endpoint and have therefore adapted the numbers in the revised appendices.*

3.4.4
Appendix 1
A. Experimentele aanpak en primaire uitkomstparameters.

1. **Vraag:** Gros van deze gedragstaken is niet geschikt voor herhaaldelijk (large-scale screening) gebruik, terwijl dat juist wel de opzet van b.v. Study 1 omvat. Hoe ondervangt U dit?
   Antwoord:
   It is true that the majority of the behavioral tests cannot be performed unremittingly, but we also did not plan to do so. We rather wanted to establish the most optimal stimulation parameter with a test, that can be done repeatedly (such as Object Location Task, Object Recognition Task, Y-Maze, Prepulse Inhibition or a modified version of the Morris Water Maze) and then test this stimulation parameter in other tasks. We have added this information to the corresponding appendix accordingly. As for large-scale screening with an AD rat model, we will evaluate behavioral parameters at certain time intervals. For example, when looking at anxiety measures, we will always keep two identical behavioral tasks a minimum of 4 weeks apart. This time interval has been employed by our group in previous studies and has been generally accepted by peer-reviewers.

D. Vervanging, vermindering en verfijning.

2. **Vraag:** Men zegt dat plaatsen van sham implantaties onethisch is bij mensen. Uiteraard correct, doch men heeft toch ook data van off-stimulatie, hetgeen hetzelfde doel kan dienen, nietwaar?
   Antwoord:
   We thank the reviewers for their valid concern. In principle this is true, however off-stimulation data in humans is always compromised by a few factors.
   As mentioned in the project proposal, the use of sham surgery in human research is controversial as it places ethical and research standards into conflict.
   While sham surgery has the potential to harm the subject, research designs without sham surgery are scientifically less rigorous. Therefore, researchers sometimes include short periods of "off-stimulation" into their stimulation paradigms.

However, all subjects with implanted DBS electrodes will be stimulated eventually, thereby making appropriate comparisons to controls, especially in long-term studies, almost impossible in human research. Therefore, sham-controlled studies are very rarely performed in humans and this is why the field can highly benefit from preclinical experiments.

B. De Dieren.

1. **Vraag:** Waar is de vehicle-controle vergelijking voor iedere behandelde (drug) groep terug te vinden? Er lijkt nu slechts 1 vehicle-groep (absolute WT controle) te worden gehanteerd, nietwaar?
   Antwoord:
   The reviewers are correct. We have included a vehicle-control group for the different DBS groups.

   - Datum antwoord 29-11-2016
   - Verstrekte antwoorden: Zie hierboven
   - De antwoorden hebben geleid tot aanpassing van de aanvraag.

10. **Eventuele adviezen door experts:** (niet lid van de DEC-UM) ***N.V.T.***

B. Beoordeling (adviesvraag en behandeling)

1. Is het project vergunningplichtig (dierproeven in de zin der wet)? Indien van toepassing, licht toe waarom het project niet vergunningplichtig is en of daar discussie over geweest is. ***JA***

2. De aanvraag betreft een **nieuwe** aanvraag.

3. Is de DEC competent om hierover te adviseren? ***Ja***

4. Geef aan of DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, zijn uitgesloten van de behandeling van de aanvraag en het opstellen van het advies. Indien van toepassing, licht toe waarom. ***N.V.T.***

C. Beoordeling (inhoud)

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft.

   Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. Het is helder welke handelingen en ongerief individuele dieren zullen ondergaan.
   De DEC-UM vertrouwt erop dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. Gezien bovenstaande is de DEC-UM van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.

2. Geef aan of er aspecten in deze aanvraag zijn die niet in overeenstemming zijn met wet- en regelgeving anders dan de Wod? Denk hierbij aan bijvoorbeeld de Flora en fauna wet en Wet dieren. Indien van toepassing, leg uit om welke aspecten het gaat en waarom hier sprake van is.

   N.v.t., daar dit buiten de taakstelling van de DEC valt overeenkomstig artikel 18a.2.b van de Wod.

3. Beoordeel of de in de projectaanvraag aangekruiste doelcategorie(ën) aansluit(en) bij de hoofddoelstelling. Nevendoelstellingen van beperkt belang hoeven niet te worden aangekruist in het projectvoorstel.

   Het projectvoorstel heeft inderdaad kenmerken van zowel fundamenteel als translationeel onderzoek.

   Belangen en waarden

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een reële relatie is tussen beide doelstellingen.

   Zie antwoord op vraag C5.

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

   De belangrijkste belanghebbenden in dit fundamenteel en toegepast wetenschappelijke project, dat gericht is op inzicht in deep brain stimulation (DBS) als mogelijke behandeling van geheugenverlies, zijn de proefdieren, de onderzoekers, de doelgroep/patiënten en hun naasten, en ook de medische wetenschap en de samenleving als geheel.
   Waarden die voor de proefdieren in het geding zijn: De integriteit van de dieren zal worden aangetast door de experimentele handelingen, de individuele huisvesting, het leven met elektroden in de hersenen en de opoffering aan het eind van de proeven. Het merendeel van de proefdieren zal matig ongerief ondervinden, voor de overige dieren wordt het ongerief geclassificeerd als non-recovery.
   Waarden die voor de onderzoekers bevorderd worden: De onderzoekers vergaren kennis over de achterliggende mechanismen en de effecten van DBS op het geheugen.
   De onderzoekers zullen medisch-wetenschappelijke kennis verkrijgen die relevant is in het onderzoek naar de ziekte van Alzheimer (AD) en deze kennis delen met de wetenschappelijke gemeenschap.
   Waarden die voor patiënten bevorderd worden: Uiteindelijk kan meer kennis over de mogelijke toepassing van DBS bij AD leiden tot verbeterde therapeutische mogelijkheden. Daardoor zou de kwaliteit van leven van deze patiënten en hun naasten verbeterd kunnen worden.
   Groei van medische kennis op een gebied waar daaraan behoefte is, wordt eveneens bevorderd door het onderhavige onderzoek. AD komt in onze samenleving steeds vaker voor en er is behoefte aan werkzame en veilige therapieën op dit gebied. Daarom heeft dit onderzoek ook belang voor de samenleving als geheel.

6. Geef aan of er sprake kan zijn van substantiële milieueffecten. Zo ja, benoem deze, leg uit waarom daar sprake van kan zijn en of geef aan of deze effecten afgedekt worden door specifieke wetgeving.

   N.v.t., daar dit buiten de taakstelling van de DEC valt overeenkomstig artikel 18a.2.b van de Wod.

   Proefopzet en haalbaarheid

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

   Voor zover de DEC-UM kan beoordelen zijn de kennis en kunde van de onderzoeksgroep adequaat gezien de wetenschappelijke output, de verworven interne- en externe financiering alsmede de aandacht voor de drie V's.

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

   De DEC-UM is er van overtuigd dat het projectvoorstel aansluit bij recente wetenschappelijke inzichten en geen hiaten bevat die de bruikbaarheid van de resultaten in de weg zullen staan. De voorgestelde experimentele opzet en uitkomstparameters zijn logisch en helder gekozen en sluiten aan bij de aangegeven doelstellingen en de gekozen strategie en experimentele aanpak kunnen naar de mening van de DEC-UM leiden tot het behalen van de doelstelling in het kader van het project.

   Welzijn dieren

9. Geef aan of er sprake is van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren. Beoordeel of de keuze hiervoor voldoende wetenschappelijk is onderbouwd en de aanvrager voldoet aan de in de Wod voor de desbetreffende categorie genoemde beperkende voorwaarden. Licht uw antwoord toe. ***N.V.T.***

10. Geef aan of de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de richtlijn. Indien niet aan deze minimale eisen kan worden voldaan omdat het om wetenschappelijke redenen noodzakelijk is hiervan af te wijken, beoordeel of dit in voldoende mate is onderbouwd. Licht toe waarom wel/niet.

    De DEC-UM heeft zich ervan verzekerd dat zulks het geval is.

11. Beoordeel of het ongerief als gevolg van de dierproeven realistisch is ingeschat en geclassificeerd, waarbij uitgegaan wordt van de kans op angst, pijn, stress en/of ziekte bij individuele dieren.

    De DEC-UM vertrouwt erop dat de aanvrager al het mogelijke zal doen om het eventuele ongerief voor de proefdieren te identificeren, te verminderen en waar mogelijk te voorkomen.

12. Geef aan op welke wijze de integriteit van de dieren wordt aangetast.

    De integriteit van de dieren zal worden aangetast door de experimentele handelingen, de individuele huisvesting en het leven met elektroden in de hersenen. De dieren worden aan het eind van de proef opgeofferd.

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

    Naar de mening van de DEC-UM zijn de humane eindpunten zorgvuldig beschreven en is de inschatting van het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken eveneens zorgvuldig beschreven in de projectaanvraag.

14. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte vervangingsalternatieven zijn? Onderbouw uw antwoord.

15.

De DEC-UM is van mening dat de doelstellingen van de proef niet behaald kunnen worden, anders dan met de aangevraagde dieren, daar geschikte vervangingsalternatieven ontbreken, zoals beschreven in onderhavig projectvoorstel.

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

    Naar de mening van de DEC-UM is het aantal te gebruiken dieren realistisch ingeschat en wel zodanig dat niet meer dan nodig, maar ook niet minder dan nodig dieren worden gebruikt voor het behalen van een betrouwbaar wetenschappelijke resultaat zulks mede gebaseerd op statistische analyse middels een poweranalyse.

17. Beoordeel of het project in overeenstemming is met de vereiste van verfijning van dierproeven en het project zodanig is opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd? Licht uw antwoord toe.

    De DEC-UM heeft zich ervan verzekerd dat de aanvrager al het mogelijke zal doen om het eventuele ongerief voor de proefdieren te identificeren, te verminderen en waar mogelijk te voorkomen. Hierbij heeft de DEC-UM onder andere de pijnbestrijding en huisvesting in haar beoordeling betrokken.

18. Beoordeel, indien het wettelijk vereist onderzoek betreft, of voldoende aannemelijk is gemaakt dat er geen duplicatie plaats zal vinden en of de aanvrager beschikt over voldoende expertise en informatie om tijdens de uitvoering van het project te voorkomen dat onnodige duplicatie plaatsvindt. Onderbouw uw antwoord.

    Voor zover de DEC-UM kan beoordelen zijn de kennis en kunde van de onderzoeksgroep adequaat en mede gezien het daartoe strekkende antwoord van de aanvrager in de projectaanvraag heeft de DEC-UM reden aan te nemen dat onnodige duplicatie achterwege blijft.

    Dieren in voorraad gedood en bestemming dieren na afloop proef

19. Geef aan of dieren van beide geslachten in gelijke mate ingezet zullen worden. Indien alleen dieren van één geslacht gebruikt worden, beoordeel of de aanvrager dat in voldoende mate wetenschappelijk heeft onderbouwd? Geef ook aan welke maatregelen verder zijn getroffen om bij fok of aankoop van dieren het aantal in voorraad gedood te beperken.

    *In onderhavige projectaanvraag worden dieren van een **eenvormig** geslacht gebruikt. Alhoewel de DEC-UM vermindering van proefdieren in voorraad gedood toejuicht is zij overigens van mening dat dit aspect met name met de centrale dienst proefdieren en de aanvrager kortgesloten dient te worden daar de DEC-UM niet betrokken is bij de fok en aankoop van proefdieren.*

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

    Naar de mening van de DEC-UM is dit genoegzaam beschreven in de projectaanvraag door de aanvrager.

21. Indien dieren worden gedood, is adoptie of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is.

    Adoptie is ten aanzien van onderhavige aanvraag niet opportuun daar het hier niet handelt om niet-humane primaten, honden, katten of landbouwhuisdieren.

    NTS

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

    Naar de mening van de DEC-UM is zulks het geval.

D. Ethische afweging

1. Benoem de centrale morele vraag.

Rechtvaardigt het verkrijgen van inzicht in het effect van DBS op geheugenverlies, de opoffering en het matige ongerief dat de dieren wordt aangedaan in het voorliggende project "Deep brain stimulation to restore memory loss?".

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

Waarden die voor de proefdieren in het geding zijn: *matig nadeel.*
Waarden die voor onderzoekers bevorderd worden: *substantieel voordeel.*
Waarden die voor de doelgroep bevorderd worden: *matig voordeel.*
Algemeen: *relevante groei van medische kennis.*

De DEC-UM is van mening dat de belangen van de samenleving in het algemeen en de patiënten en hun naasten in het bijzonder binnen het project "Deep brain stimulation to restore memory loss" zwaarder wegen dan de belangen/waarden van de proefdieren. Voor de betrokken proefdieren leiden deze proeven, na voornamelijk matig ongerief, tot de dood. Zij worden door de experimenten in hun welzijn geschaad. De integriteit van de dieren wordt geschaad door de experimentele handelingen, de individuele huisvesting, het leven met elektroden in de hersenen en de opoffering aan het eind van de proeven. Indien de doelstellingen bereikt worden, zal dit project echter leiden tot kennis over het effect van DBS op geheugenverlies en eventuele bijwerkingen, waarbij verschillende stimulatieparameters worden gevarieerd, zoals frequentie en duur van stimulatie. Tevens zal er kennis zijn verkregen over de onderliggende mechanismen van DBS en de betreffende bijwerkingen. Ten slotte zal er kennis zijn verkregen over de effecten van verschillende neurofarmaca in combinatie met DBS in de behandeling van geheugenverlies in een Alzheimer diermodel.
De verwachting is dat de verworven inzichten op termijn bouwstenen kunnen leveren voor een betere behandeling van Alzheimer patiënten. AD is een veel voorkomende aandoening, die voor de patiënt een hoge ziektelast en zorgbehoefte met zich meebrengt en ook voor de naasten zeer belastend is. Het huidige therapeutische arsenaal is beperkt. Door een verbeterde therapie van AD zou uiteindelijk de kwaliteit van leven verbeterd kunnen worden van grote aantallen patiënten en hun naasten.
Daarnaast is passende zorg voor deze categorie patiënten tijdrovend en kostbaar. Dit onderzoek heeft daarom ook belang voor de samenleving als geheel. Vandaar dat de DEC-UM het onderhavige onderzoek van substantieel belang acht.

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD107002016785
Bijlagen
1

16 FEB. 2017

Datum 15 februari 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 15 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Deep brain stimulation to restore memory loss" met aanvraagnummer AVD107002016785. Wij hebben uw aanvraag beoordeeld.

Op 3 februari 2017 en 13 februari 2017 heeft u uw aanvraag gewijzigd. Wij hebben u om aanvullende informatie gevraagd over het vervoer van dieren tijdens de proef. U geeft aan dat er tussen de operatie en eventueel vervoer circa 12 weken zit, dat alleen dieren die gezond genoeg zijn vervoerd zullen worden en dat dieren na transport 5 dagen de tijd krijgen om te acclimatiseren. Wij kunnen ons hierin vinden.

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

U kunt met uw project "Deep brain stimulation to restore memory loss" starten. De vergunning wordt afgegeven van 15 februari 2017 tot en met 1 februari 2022.

Overige wettelijke bepalingen blijven van kracht.

Beoordeling achteraf
Na afloop van het project zal er een beoordeling plaatsvinden, zoals bedoeld in artikel 10a1, lid 1d en lid 3, in de wet. Meer informatie over de eisen bij

een beoordeling achteraf vindt u in de bijlage.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC-UM gevoegd. Dit advies is opgesteld op 15 december 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 8 februari 2017 heeft de DEC gereageerd op onze vragen. De DEC heeft ons aanvullend geadviseerd over eventuele strijdigheid met andere wetgeving, mogelijke milieueffecten, de relatie tussen het directe doel en het indirecte doel, de relatie tussen het directe doel en de status van het onderzoeksveld en de onderbouwing van het toepassen van huisvesting anders dan volgens bijlage III van de richtlijn.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

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

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

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

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

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

Centrale Commissie Dierproeven
namens deze:
[redacted]

ir. G. de Peute[redacted]
Algemeen Secretaris

Datum:
15 februari 2017
Aanvraagnummer:
AVD107002016785

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

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

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

deze projectvergunning voor het tijdvak 15 februari 2017 tot en met 1 februari 2022, voor het project "Deep brain stimulation to restore memory loss" met aanvraagnummer AVD107002016785, volgens advies van Dierexperimentencommissie DEC-UM. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is [redacted]. Voor de uitvoering van het project is Post-Doctoral researcher verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 15 december 2016
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 15 december 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 15 december 2017;
c Advies van dierexperimentencommissie d.d. 15 december 2016, ontvangen op 15 december 2016.
d De aanvullingen op uw aanvraag, ontvangen op 3 februari 2017 en 13 februari 2017

Aanvraagnummer:
AVD107002016785

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1. The effects of fornix and nucleus basalis DBS on cognition, behavior and neuronal networks				
	Ratten (Rattus norvegicus) /	84	100% Matig	
3.4.4.2. Changes in neurotransmitters and brain activation induced by acute and chronic fornix and nucleus basalis DBS				
	Ratten (Rattus norvegicus) /	280	25% Terminal 75% Matig	
3.4.4.3. Long-term DBS in an animal model of disease				
	Ratten (Rattus norvegicus) /	216	100% Matig	
3.4.4.4. The role of neuropharmacology in DBS for dementia-related disorders				
	Ratten (Rattus norvegicus) /	416	100% Matig	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen
Dit project wordt voorzien van beoordeling achteraf.Deze beoordeling zal uiterlijk februari 2023 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.

Aanvraagnummer:
AVD107002016785

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

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

Voorschriften
Dieren mogen gedurende een proef niet verplaatst worden. Indien, vanwege het niet beschikbaar zijn van een MRI scanner in de eigen instelling, dieren naar een andere locatie vervoerd moeten worden, moet de hele proef op de andere locatie uitgevoerd worden.

Aanvraagnummer:
AVD107002016785

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.

Locatie
De vergunning wordt verleend voor een project waarbij dierproeven geheel of gedeeltelijk worden verricht buiten een inrichting van een gebruiker (artikel 10g van de wet).

Beoordeling achteraf
Volgens artikel 10a1, lid 1d en lid 3 van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden.

Centrale Commissie Dierproeven

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Onze referentie
AVD107002016785

Uw referentie

Bijlagen
1

Datum 15 maart 2017
Betreft Correctie beslissing Aanvraag projectvergunning dierproeven

Geachte [redacted]

Op 15 december 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Deep brain stimulation to restore memory loss" met aanvraagnummer AVD107002016785.

Beslissing
Op 15 februari 2017 heeft u de beschikking en vergunning van uw aanvraag ontvangen.
In de beschikking staat vermeld dat wij ons kunnen vinden in uw toelichting over het vervoer van dieren tijdens de proef. Uit uw toelichting blijkt namelijk dat er tussen de operatie en eventueel vervoer 12 weken zit, dat alleen dieren die gezond genoeg zijn vervoerd zullen worden en dat dieren na transport 5 dagen de tijd krijgen om te acclimatiseren.
In de vergunning is echter het volgende voorschrift opgenomen: "Dieren mogen gedurende een proef niet verplaatst worden. Indien, vanwege het niet beschikbaar zijn van een MRI scanner in de eigen instelling, dieren naar een andere locatie vervoerd moeten worden, moet de hele proef op de andere locatie uitgevoerd worden."
Dit voorschrift is ten onrechte in uw vergunning opgenomen.

De aan u verstuurde vergunning bevat dus een kennelijke verschrijving en moet gecorrigeerd worden. Bovengenoemd voorschrift komt hierbij te vervallen. Voor het overige blijft het besluit van 15 februari 2017 ongewijzigd.
Deze brief dient u bij uw vergunning te voegen.

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

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

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

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

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

De Centrale Commissie Dierproeven
namens deze:

[redacted]

Ir. G. de Peuter
Algemeen Secretaris

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