		Inventaris Wob-verzoek W17-09								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 20171327**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Aanvraagformulier				x		x	x	
2		NTS oud			x					
3		NTS nieuw	x							
4		Projectvoorstel			x					
5		Bijlage beschrijving dierproeven 1 en 2 oud			x					
6		Bijlage beschrijving dierproeven 1 en 2 nieuw			x					
7		Ontvangstbevestiging en factuur				x		x	x	
8		DEC advies				x		x	x	
9		Verzoek en antwoord aanvullende informatie				x		x	x	
10		Mail verzoek en antwoord aanvullende informatie				x		x	x	
11		Mail verzoek en antwoord aanvullende informatie DEC				x		x	x	
12		Advies CCD		x						x
13		Beschikking en vergunning				x		x	x	

AVD 10300 2017 1327

1.

11 APR. 2017

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

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

1 Gegevens aanvrager

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

☑ Ja > *Vul uw deelnemernummer in* 10300

☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	Stichting Katholieke Universiteit Nijmegen
Naam van de portefeuillehouder of diens gemachtigde	Instantie voor dierenwelzijn
KvK-nummer	4 1 0 5 5 6 2 9

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

Straat en huisnummer	Geert Grooteplein 29
Postbus	9101, [redacted]
Postcode en plaats	6500HB Nijmegen
IBAN	NL90ABNA0231209983
Tenaamstelling van het rekeningnummer	UMC St Radboud

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	Instantievoor Dierenwelzijn
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: 08-05-2017 Einddatum: 07-05-2022
3.2 Wat is de titel van het project?	Alzheimer's disease and hyperactivity
3.3 Wat is de titel van de niet-technische samenvatting?	Hyperactiviteit en de ziekte van Alzheimer
3.4 Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC: RU DEC Postadres: Postbus 9101, 6500 HB Nijmegen[redacted] E-mailadres: [redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

- ☑ Nieuwe aanvraag Projectvergunning € 1.287,00 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
- ☒ DEC-advies en factuurinformatie

6 Ondertekening

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

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.6). De ondergetekende verklaart:

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

Naam	[redacted]
Functie	Instantie voor dierenwelzijn
Plaats	Nijmegen
Datum	0 7 - 0 4 - 2 0 1 7
Handtekening	[redacted]

2.

Format
Niet-technische samenvatting

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

1 Algemene gegevens

1.1	Titel van het project	Hyperactiviteit en de ziekte van Alzheimer
1.2	Looptijd van het project	8-5-2017 - 7-5-2022
1.3	Trefwoorden (maximaal 5)	Alzheimer, farmaceutische manipulatie, epilepsie

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

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

3 Projectbeschrijving

3.1	Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)	Er lijden per jaar 48 miljoen mensen aan de ziekte van Alzheimer, een ernstige geheugenstoornis. Dit aantal zal waarschijnlijk toenemen tot 130 miljoen in 2050. Momenteel is het alleen mogelijk om de symptomen te bestrijden en bestaat er geen behandeling die de oorzaken van deze ziekte bestrijdt. Er zijn aanwijzingen dat hersenen van mensen met de ziekte van Alzheimer te makkelijk geprikkeld zijn, net zoals bij mensen met epilepsie. Wij zullen netwerken van hersencellen bestuderen die betrokken zijn in geheugenprocessen en analyseren of er sprake is van abnormale activiteit zoals die ook bij epilepsie optreedt. Ook verdiepen we ons in de effectiviteit van nieuwe behandelingen voor patiënten met epilepsie, zoals geneesmiddelen en elektrische stimulatie. We onderzoeken of die ook geschikt zijn om te voorkomen dat het geheugen achteruit gaat.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	De uitkomst van dit onderzoek zal helpen de ziekte van Alzheimer eerder te detecteren met behulp van biomarkers (electroencephalography en magnetoencephalography). Ook zullen de resultaten van het onderzoek mogelijk bijdragen aan het ontwikkelen van nieuwe behandelingen voor Alzheimer die de oorzaken van de ziekte aanpakken, zoals betere geneesmiddelen en diepe hersenstimulatie.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	We zullen maximaal 1170 muizen gebruiken. licht ongerief 34 % matig ongerief 36% ernstig ongerief 30 %
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	Transgene dieren zouden ongerief kunnen hebben vanwege epileptische aanvallen. Dieren zullen chirurgische ingrepen ondergaan die nodig zijn voor het implanteren van apparatuur voor het meten en beïnvloeden van neurale activiteit. Geïmplanteerde dieren moeten individueel worden gehuisvest om schade te voorkomen aan het implantaat en andere negatieve gevolgen voor het welzijn van het dier. Dieren zullen gedragstaken uitvoeren waarbij het ongerief licht zal zijn.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Het ongerief zal matig zijn voor alle dieren.

3.6	Wat is de bestemming van de dieren na afloop?	Dieren zullen gedood worden na afloop van de experimenten zodat de hersenen onderzocht kunnen worden.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Deze experimenten zijn ontworpen om hersenactiviteit aan cognitie en gedrag te koppelen. Om deze redenen zijn levende dieren nodig. De beschikbare data zijn niet voldoende om deze wetenschappelijke vragen te beantwoorden met behulp van computermodellen.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	We gebruiken technologieën met een hoog rendement waardoor we veel data per dier kunnen verzamelen en het aantal benodigde dieren beperkt blijft. We zullen niet meer dieren gebruiken dan noodzakelijk om betrouwbare conclusies te kunnen trekken.
4.3	**Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Muizen worden gebruikt omdat het de kleinste, genetisch aanpasbare, zoogdieren zijn waar dit onderzoek in mogelijk is. We maken gebruik van extra kleine implantaten die zo min mogelijk invloed hebben op het welzijn en de bewegingsvrijheid van het dier. De technieken die wij gebruiken zijn zeer modern en verfijnd.
4.4	Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	Alle operaties worden uitgevoerd met de beste werkmethoden op het gebied van anesthesie, pijn management en post-operatieve zorg. Ook houden wij dagelijks de gezondheid en het welzijn van de dieren in de gaten en huisvesten we de dieren in een verrijkte omgeving. Wanneer een dier meer ongerief heeft dan toegestaan voor dit onderzoek dan zal het worden gedood om onnodig lijden te voorkomen.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	

4.

Form
Project proposal

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen
1.3	Provide the title of the project.	Alzheimer's disease and hyperactivity

2 Categories

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

[X] Basic Research
[X] Translational or applied research
[] Regulatory use of routine production
[] Research into environmental protection in the interest of human or animal health or welfare dier
[] 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.

Alzheimer's disease (AD) related dementia, memory disorder, affects 48 million people globally each year. This number will almost double every 20 years estimated to reach 130 million Alzheimer patients by 2050 (1). Devastatingly current treatments are only able to alleviate the symptoms but not to provide permanent cure emphasizing the importance of finding novel treatment targets and biomarkers for more accurate and earlier detection and treatment of the disease.

Novel exciting findings have raised the possibility that Alzheimer disease could actually be caused by abnormal excitatory neuronal activity leading to cognitive and behavioral deficits common in AD (2–4). Clinical observations support this idea that such abnormal activity may play role also in the AD patients. It has been shown that patients with strong familiar genetic risk of AD (mutations in presenilin-1, presenilin-2, or the amyloid precursor protein, or with duplications of wild-type amyloid precursor protein) also express even tenfold risk of epileptic seizures and patients with non-familiar (inheritance of apolipoprotein E4) form of AD are more prone to express induced seizures(5). Importantly, this hyperactivity can be observed at the early stage of the disease, already at the Mild Cognitive Impairment (MCI) stage being one the earliest clinical biomarkers of the pathology. This form of neuronal hyperactivity can be observed in EEG as epileptiform activity including simple parietal, complex parietal or generalized seizures with wide range of behavioural changes such as single limb shaking, altered consciousness, speech arrest and /or atony (6). However, it should be noted that AD linked hyperactivity has been also showed in MCI patients in the hippocampal area by using functional magnetic resonance imaging (fMRI) (7). Importantly, antiepileptic drug levetiracetam is able to decrease observed hyperactivity and slightly improve the memory decline at this stage both in patients and in animal models (7,8). Even though these highly exciting and promising results, these findings can only be considered as preliminary leaving a lot of open question. More studies are needed to resolve e.g. which brain areas are most susceptible to hyperactivity, which subtypes of neurons are affected by hyperactivity or /and are the locus of epileptic activity, what are the mechanisms of efficient antiepileptic drugs and could hyperactivity be controlled by other more efficient methods?

In this project we investigate the link between the abnormal single cell and neuronal activity, and memory and cognitive deficits in mice carrying human APPswe and PS1dE9 (APdE9) (9). Because of mutations these mice develop amyloid plaques beginning at 4-5 months of age and show memory impairment in memory tasks around 10-12 months of age (10,11). Interestingly, these mice show increase in epileptic seizures even before

actual amyloid plaque accumulation in the age between 3 and 4.5 months reaching eventually a prevalence of 60% of the population (3). At this age, as we have shown, mice also display cortical and-thalamic hyperactivity implying this neuronal network be first one affected by the accumulating amyloid load (4). Therefore, in this project we will characterize how hyperactivity and epileptiform activity related to amyloid pathology affects the two memory-related networks, the corticothalamic and the intra-hippocampal networks in single cell and in neuronal network level. Based on the characterization we will try to provide causal evidence on the link between hyper excitability and cognitive decline by using pharmacological, pharmacogenetical, optogenetical stimulation and electrical or ultrasound stimulation.
To summarize, the focus of this project is to characterize the link between electrical activity and memory. This is an aspect of AD that has been so far under investigated.

3.2 Purpose

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

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

The main objectives of this researchare
1.

Categorize how hyperactivity and epileptiform activity related to amyloid pathology affects the two memory-related networks, the corticothalamic and the intra-hippocampal networks in single cell and in neuronal network level. This is done in young animal (3-4 months) and in old animals (12-18 months). The young animals are more prone to even severe epileptiform hyperactivity but the cognitive decline is only detectable in older animal, mimicking closely clinical findings.
2. To reverse observed hyperactivity in these networks and characterize the unknown mechanisms of action pharmacological, optogenetical, pharmacogenetical manipulation and/or electrical stimulation.
3. Based on the results of previous work packages we will conduct behavioral tests to verify that suppression of pathological hyperactivity improves cognitive functions.

This project is a coherent continuum of our previous work concentrated on hyperactivity in AD animal models, and this project is supported by extensive co-operation by other international groups having years of experience on this specific issue and working with animals (3,4,11). Additionally, the lab is qualified to carry out this research project, based on our track record of producing significant advance in this field, our ability to use the most advanced techniques (12–14). We propose a plan of closely related experiments, involving neural recordings and perturbation of neural activity in awake, behaving rodents. Furthermore, we are continuously working at improving our experimental technology, in collaboration with bioengineering and neuroengineering groups. This in order to obtain, larger, more precise data sets to best address the specific research questions in this project, and to improve animal welfare.

3.3 Relevance

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

Alzheimer's disease related dementia, memory disorder, affects 48 million people globally each year, and the number of patients are estimated to reach 130 million Alzheimer patients by 2050. Devastatingly current treatments are only able to alleviate the symptoms but not to provide permanent cure. So, there is desperate need to develop and identify novel targets for treatments but also biomarkers, e.g. EEG and/or MEG markers, allowing earlier detection of the disease.

Overall this project concerning the hypo- or/and hyperactivity of single neurons and aberrant network activity related to progressive amyloid pathology provides a ground breaking platform to identify novel biomarkers (epileptiform activity detected by EEG or MEG) for early detection of AD in patients but also for innovative disease-modifying targets. However, the findings will also benefit the field of epilepsy research by providing novel mechanisms of epileptogenesis in the medial temporal lobe and cortico-thalamic related pathologies. In addition, the proposed intracranial electrical stimulation provides a novel mechanisms of clinical deep brain stimulation currently being tested as for a treatment for AD.

3.4 Research Strategy

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

The research project will consist in a set of neurophysiological, behavioral and manipulation experiments in amyloid expressing transgenic and wild type control mice. For the most part animals will be awake during the experiments, and engaged in behaviors that entail the encoding, or the retrieval of memories (equivalent to what would be called in humans 'declarative memories'). Experiments will also follow neural activity sleep and other inactive behavioral, in order to characterize the memory consolidation phase.

We aim at collecting a data set on neural activity during memory acquisition, consolidation and retrieval, linking the neural circuit level to cognitive performance, and representing the basis for a new theory of cognitive decline related to progressive amyloid pathology. Additionally, the neural activity is related to the efficacy of the treatments to reveal potential new drug targets and novel biomarkers.

Animal model for amyloid related Alzheimer pathology

The hypotheses will be tested with mice carrying human APPswe and PS1dE9 (APdE9) mutations which develop amyloid plaques beginning at 3-4 months of age without memory dysfunction. These animals show memory impairment in memory tasks around 10-12 months of age when these animals also suffer from severe amyloid plaque burden (9–11). These mice show increase in epileptic seizures between 3 and 4.5 months of age reaching eventually a prevalence of 60% of the population (3). At this age, as we have shown, mice also display cortical and-thalamic hyperactivity (4). Therefore, studying the young animals may lead to the detection of early EEG biomarkers for early stages of AD, and studies with older animals to the characterization of the pathology of the cognitive loss.

Based on our previous studies and other literature we will concentrate on the two memory-related networks, the corticothalamic e.g. sensory cortex – reticulate thalamic nucleus - thalamus and the hippocampal-cortical networks e.g entorhinal cortex – hippocampus -subiculum. These neuronal networks are also prone to hyperactivity and amyloid pathology in this animal model (4,9,15–18).

Experimental techniques will consist of the following components, which will provide complementary data on the same research data:
Measurement techniques

- Massive extracellular electrophysiology for recording large ensembles of neurons in multiple areas, to characterize and decode the precise temporal structure of neural network activity. This allow simultaneous observations in single cell and neuronal network level to determine the state and locus of hyperactivity and the effect and mechanisms of treatments.
- Calcium imaging enables to sample even larger number of single cell activity in specific brain areas, albeit with lesser temporal resolution.
- Large scale characterization of global brain activity with e.g. electrocorticography for detecting electrical activity in multiple cortical areas

Interventional techniques

- Optogenetical controlling of specific subpopulations of neurons with millisecond temporal scale
- Pharmacological and chemogenetic approaches allowing specific control of neurotransmitter systems to control hyperactivity
- Electrical deep brain stimulation to control and restore of activity in neuronal networks

Behavioral techniques

- Automated mazes and behavioral setups for freely moving experiments
- Head fixed virtual reality

These methods will be employed in combination (or sometimes as alternative to find out the best experimental setup) in order to address the research questions delineated above.
The experiment will be sub-divided in a number of groups of relatively small size (actual group size will be determined by power analysis, but each group will usually have a max. size of 20.
Group sizes will be kept small thanks to the large yield in recorded neurons allowed by our techniques.

Techniques for readout and manipulation of brain activity

To study how these complex, brain-wide patterns of activity link to memory, behavior and related pathologies, it is necessary to get a handle on the activity of large numbers of neurons spread throughout the brain, and to manipulate them with large degree of specificity. In the last years, there has been an explosion of new techniques that allow us to observe neural activity, and to manipulate brain activity at unprecedented scale and resolution. These techniques constitute the ‘toolbox’ of a modern systems neuroscience laboratory, and are applied singularly, or most productively, in creative combinations.

The techniques the laboratory has experience with and we intend to apply in this project, in different combinations are:

Readout technologies:

- CMOS based silicon probes. These probes are manufactured in silicon with techniques similar to those used to make computer chips. They are intensively miniaturized and they enable recording of electrical potential from a large number (up to thousands) of sites in the brain, while maintaining a form factor that is still compatible with comfortable use even in mice.
- Flexible support electrode arrays. These electrode types, similar to those used in human (e.g. epileptic) patients, enable novel recording configurations, for example from a large number of cortical sites with ElectroCorticoGraphy (ECoG) grids.
- Calcium imaging of neural activity. By combining genetically encoded calcium indicators (GCamp proteins) expressed by viral transfection, and advanced microscopy techniques (such as two-photon microscopy) it is possible to record the activity of thousands of neurons, albeit at a time resolution slower than what is allowed by electrophysiology. Recently, miniaturized 'wearable' microscopes have been devised that enable use in freely-moving mice.

Activity Manipulation Techniques

- Optogenetics. By genetically inserting (via a viral transfection) light-sensitive molecules in the neuronal membrane, it is possible to control neural activity of specific neuronal population in a very precise way.
- Electrical stimulation. Delivering mild electrical deep brain stimulation it is possible to balance the observed hyperactivity of neuronal tissue in millisecond scale
- Pharmacology. Antiepileptic and/or hyperactivity reducing drugs affect to the brain areas affected by AD pathology and restore abnormal neuronal activity
- Advanced pharmacology. Viral vectors can also be used to express in selected neural populations designer receptors (Designer Receptors Exclusively Activated by Designer Drugs, DREADDs), that are activated by designer drugs (molecules that are not normally present in the brain). These chemogenetical methods enable long-lasting, specific manipulations of certain neuronal populations and their activity.

These techniques will be employed in the context of:

- Behavioral tasks. The severity of AD pathology and the efficacy of manipulations are determined in behavioral task sensitive to amyloid pathology and effects of manipulations.
- Novel behavioral paradigms. An example of innovation in this field is the 'Virtual reality' setup where rodents are head fixed but can run on a track ball, or wheel, or other device while projection screens and other sensory stimulation devices provide the illusion of moving in a virtual environment. Animal can be tested with bulkier setups (e.g. microscopes) that do not lend themselves to implant in freely-moving animals.

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

The project has 3 major workpackages based on the objectives (see also section 3.2.). Each workpackages of the experiment is optimized by utilizing the results of the previous stage allowing us to enhance the workflow of the project. The three major workpackages (see table 1). The experiments are conducted in male mice. The scope of this research is not in the role of hormonal effect on the AD pathology. Because there is gender differences in amyloid burden accumulation and overall AD pathology is strongly linked to the fluctuation in the estrous cycle, we won't use female mice (24, 25).

1) To detect the neuronal activity animals are implanted during anesthesia with a carrier for electrodes for electrophysiological recordings, or with a lens allowing calcium imaging. Mice are implanted during anesthesia with electrodes allowing to observe electrical activity of single neurons and neuronal network activity in targeted brains areas related to memory and cognitive functions. Young (3-4 months) and old (12-14 months) AD mice, and age matched wild type controls are chronically implanted during anesthesia with electrodes allowing to observe electrical activity of single neurons and neuronal network activity in targeted brains areas related to memory and cognitive functions. After recovery period the animals are allowed to move freely in their home cages while video EEG is recorder.
2) Animals are subjected to pharmacological, optogenetical, pharmacogenetical or electrical manipulation to restore and/or prevent abnormal neuronal activity. In the goal two the aim is to characterize the mechanism of action of different treatments for AD related hyperactivity. The animals will be treated with acute intraperitoneal injection of a low dose of clonazepam (0.05 mg/kg), or with antiepileptic drug, levetiracetam, acutely 200 mg/kg i.p., or chronically 75 mg/kg daily administered by miniosmotic pumps (e.g. Alzet miniosmotic pumps http://www.alzet.com/) for 28 days. The dosing is based on previous animal and human experiments for controlling hyperactivity in memory decline (7,8,19). Alternatively, intracortical bipolar stimulation is achieved by stimulating the deep part of the electrode in the cortex (20). Optogenetical manipulation is achieved by injecting viral vectors carrying a gene construct for light sensitive ion channel. Additionally, animals are equipped with small chronic optic cable during electrode implantation (21). Ultrasound stimulation of brain areas will be achieved by an integrated focused ultrasound system was used (22). Pharmacogenetical manipulation will be based on A DREADD, designer receptor, which will be expressed in neurons. Since the HM4Di has no natural ligands and respond exclusively to a synthetic small molecule, clozapine N-oxide (CNO), administration of CNO provides us a temporally and spatially specific method to control hyperactive (23).
3)

The efficacy of treatment is determined by behavioral tasks sensitive to amyloid pathology but also to the effects of treatments. To accelerate learning a behavioral task and facilitate motivation of the animal, (partial) food or water restriction may be required. Animals will be weighed regularly and weight is maintained at ~85% of its original weight. We also compare the animals' weight with a normal growth curve to make sure an animal is maintaining a healthy weight. The animals receive 1.5-2.5 gram (~1 pellet) per day when it's on food restriction. This is excluding the amount of food the animal receives as a reward, which will vary according to the animals' performance

Table 1. The project is coherent entity aiming to reveal the neuronal mechanism behind hyperactivity as a primary mechanism of Alzheimer’s disease. At the WP1 (workpackage) animals are equipped with electrophysiological or calcium imaging equipment allowing us to record individual neurons and neuronal activity of populations of neurons. At the WP2 state-of-art manipulation methods aimed to control hyperactivity is being used and their mechanisms of action is characterized. Finally at the WP3 the progression of the disease and the efficacy of manipulations is determined in behavioral tasks.

WP	Objective	Methods		Go and no-go
1	Characterization of abnormal neuronal activity in cortico-thalamic and cortico-hippocampal networks	Implantation (only one method will apply per animal)	Electrode carrier	Basic characterization. NA
			Lenses for calcium imaging	
2	Reverse observed hyperactivity and characterize the main target of the treatment	Manipulation (only one method will apply per animal)	Pharmacological • Acute i.p. levetiracetam • Chronic s.c levetiracetam • Acute i.p. clonazepam	- We choose the target brain area and manipulation based on the results of the WP1 - If a manipulation proves to be not efficient, other manipulation will apply
			Advanced pharmacology - pharmacogenetics	
			Electrical deep brain stimulation	
			Optogenetics	
			Ultrasound	
3	Verify the improvement of cognitive function	Behavioral task (only one method will apply per animal	Freely moving animals	- We choose the manipulation to be tested based onthe results of the WP2
			Head fixed animals	

As described, we make use of freely moving and head-fixed virtual reality behavioral settings. These are described in distinct appendices because of their different animal welfare profiles. Again, these techniques are both necessary as they present specific advantages, as described in the

appendices. Briefly, head fixed setup allow us to use bulkier equipment which could not be implanted permanently on the animal's head. Also virtual reality setups enable more flexible behavioral contexts than what's possible in real environments. On the other hand, some specific types of memory, for example for space, physical objects, social recognition memory, require to be tested in freely-moving animals.

We breed the APdE9 line in-house. Breeding is done to provide animals for both experimental procedure, and will be performed jointly with another research group. In order to maintain the transgenic mouse line, breeding will start with wild type females (C57Bl6j, commercially available) and 24, 4-12 months old transgenic APdE9 males (from the lab animal facility). We will obtain around 130 mice in the first year, of which based on the previous breeding results we will obtain 80 transgenic (40 males and 40 females), and 50 wild type mice (15 males and 35 females). Because of an expected 20% loss of animals, because of epilepsy, we will be left with around 32 new transgenic breeding males. The procedure will be repeated in the following years of the project in order to obtain a sufficient number of animals. Altogether, for the breeding over 5 years we will need 400 (5x80) APdE9 transgenic mice. The animal are used for breeding purpose only until they reach age of 14 month of age after which they will be used as a late stage experimental group in the experiment. This allows us to reduce the amount animals needed to be bred for the old stage experiments, and this technique has been successfully deployed in the lab. The use of only male breeders is justified because this mouse line is maintained as a heterozygous genotype: homozygous animals have no survival chance, because the homozygous mutation makes females unfertile. Heterozygous females have low fertility and do not take care of their offspring. Therefore, we need to breed with heterozygous males and WT females. Therefore, we estimate to use 400 transgenic animals for breeding purposes depending on their breeding capabilities, and, additionally, we will use a maximum of 770 mice (each group including transgenic and control subjects), altogether 1170 mice. We note that, because of high yield, we may obtain strong statistical power with considerably smaller groups.

The animal are used for breeding purpose only until they reach age of 14 month of age after which they will be used as a late stage experimental group in the experiment. This allows us to reduce the amount animals needed to be bred for the old stage experiments, and this technique has been successfully deployed previously.
The use of only male breeders is justified because this mouse line is maintained as a heterozygous genotype: homozygous animals have no survival chance, because the homozygous mutation makes females unfertile. Heterozygous females have low fertility and do not take care of their offspring. Therefore, we need to breed with heterozygous males and WT females.

Therefore, we estimate to use 400 transgenic animals for breeding purposes depending on their breeding capabilities, and, additionally, we will use a maximum of 770 mice (each group including transgenic and control subjects), altogether 1170 mice. We note that, because of high yield, we may obtain strong statistical power with considerably smaller groups.

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

The different parts in this project will be closely intertwined, and they will look at different components of the neural circuits and manipulations, and will be based on the previous steps. The research project will consist in a set of neurophysiological, behavioral and manipulation experiments in Alzheimer's disease modeling amyloid expressing transgenic and wild type control mice aiming to reveal the neuronal mechanisms of amyloid related hyperactivity. To achieve this aim different pharmacological and other manipulation methods are applied to AD transgenic animal model mice

(APdE9) and their non-transgenic control littermates while neuronal activity is recorded by electrophysiological or calcium imaging methods. The efficacy and mechanism of treatments are verified by behavioral tests. Also they will look at different levels the effects of manipulation on neuronal and neuronal network levels and correlate it with performance in cognitive tasks to reveal how amyloid related pathology interfere with these processes (see fig. 1).

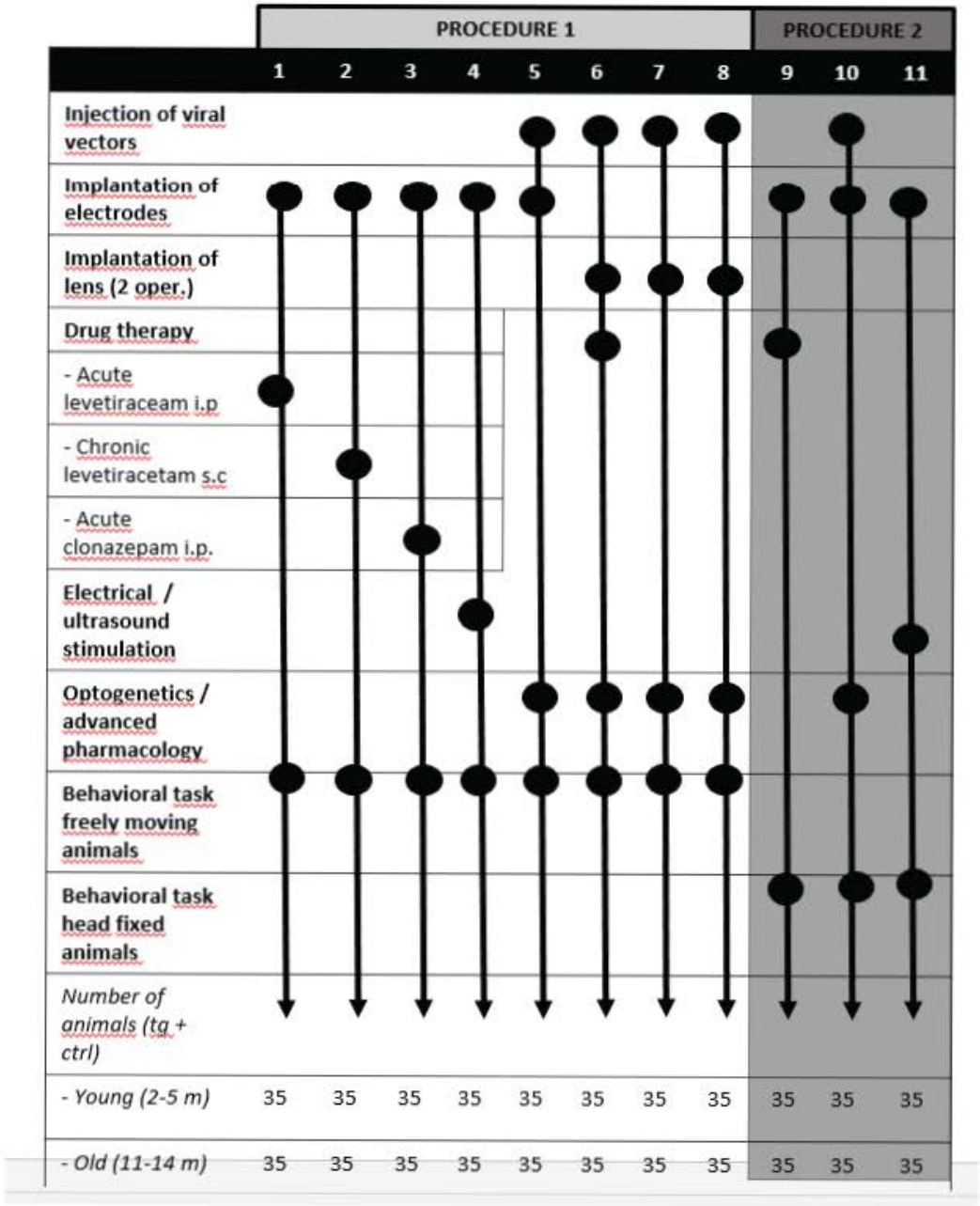
PROCEDURE 1
PROCEDURE 2
1 2 3 4 5 6 7 8 9 10 11
Injection of viral vectors
Implantation of electrodes
Implantation of lens (2 oper.)
Drug therapy
- Acute levetiraceam i.p
- Chronic levetiracetam s.c
- Acute clonazepam i.p.
Electrical / ultrasound stimulation
Optogenetics / advanced pharmacology
Behavioral task freely moving animals
Behavioral task head fixed animals
Number of animals (tg + ctrl)
- Young (2-5 m) 35 35 35 35 35 35 35 35 35 35 35
- Old (11-14 m) 35 35 35 35 35 35 35 35 35 35 35

Figure 1. A detailed description of individual animal groups and workflow. Different pharmacological and other manipulation methods are applied to AD transgenic animal model mice (APdE9) and their non-transgenic control littermates while neuronal activity is recorded by electrophysiological or calcium imaging methods. The efficacy and mechanism of treatments are verified by behavioral tests.

Bibliography

1. WHO. World Alzheimer Report [Internet]. 2015 [cited 2016 Sep 14]. Available from: http://www.worldalzreport2015.org/downloads/world-alzheimer-report-2015.pdf
2. Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, et al. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron. 2007 Sep 6;55(5):697–711.
3. Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fülöp L, et al. Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. J Neurosci Off J Soc Neurosci. 2009 Mar 18;29(11):3453–62.
4. Gurevicius K, Lipponen A, Tanila H. Increased cortical and thalamic excitability in freely moving APPswe/PS1dE9 mice modeling epileptic activity associated with Alzheimer's disease. Cereb Cortex N Y N 1991. 2013 May;23(5):1148–58.
5. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. Arch Neurol. 2009 Apr;66(4):435–40.
6. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. JAMA Neurol. 2013 Sep 1;70(9):1158–66.
7. Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, et al. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron. 2012 May 10;74(3):467–74.
8. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc Natl Acad Sci U S A. 2012 Oct 16;109(42):E2895-2903.
9. Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, et al. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. Hum Mol Genet. 2004 Jan 15;13(2):159–70.
10. Garcia-Alloza M, Robbins EM, Zhang-Nunes SX, Purcell SM, Betensky RA, Raju S, et al. Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. Neurobiol Dis. 2006 Dec;24(3):516–24.
11. Minkeviciene R, Banerjee P, Tanila H. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. J Pharmacol Exp Ther. 2004 Nov;311(2):677–82.
12. Battaglia FP, Sutherland GR, McNaughton BL. Hippocampal sharp wave bursts coincide with neocortical "up-state" transitions. Learn Mem. 2004 Nov;11(6):697–704.
13. Peyrache A, Battaglia FP, Destexhe A. Inhibition recruitment in prefrontal cortex during sleep spindles and gating of hippocampal inputs. Proc Natl Acad Sci U S A. 2011 Oct 11;108(41):17207–12.
14. Holleman E, Battaglia FP. Memory Consolidation, Replay, and Cortico-Hippocampal Interactions. In: Tatsuno M, editor. Analysis and Modeling of Coordinated Multi-neuronal Activity [Internet]. Springer New York; 2015 [cited 2016 Sep 17]. p. 207–21. (Springer Series in Computational Neuroscience). Available from: http://link.springer.com/chapter/10.1007/978-1-4939-1969-7_10
15. Dhenain M, El Tannir El Tayara N, Wu T-D, Guégan M, Volk A, Quintana C, et al. Characterization of in vivo MRI detectable thalamic amyloid plaques from APP/PS1 mice. Neurobiol Aging. 2009 Jan;30(1):41–53.

16. Darvesh S, Cash MK, Reid GA, Martin E, Mitnitski A, Geula C. Butyrylcholinesterase is associated with β-amyloid plaques in the transgenic APPSWE/PSEN1dE9 mouse model of Alzheimer disease. J Neuropathol Exp Neurol. 2012 Jan;71(1):2–14.
17. Beenhakker MP, Huguenard JR. Neurons that fire together also conspire together: is normal sleep circuitry hijacked to generate epilepsy? Neuron. 2009 Jun 11;62(5):612–32.
18. Destexhe A, Contreras D, Steriade M. Mechanisms underlying the synchronizing action of corticothalamic feedback through inhibition of thalamic relay cells. J Neurophysiol. 1998 Feb;79(2):999–1016.
19. Busche MA, Kekuš M, Adelsberger H, Noda T, Förstl H, Nelken I, et al. Rescue of long-range circuit dysfunction in Alzheimer's disease models. Nat Neurosci. 2015 Nov;18(11):1623–30.
20. Maingret N, Girardeau G, Todorova R, Goutierre M, Zugaro M. Hippocampo-cortical coupling mediates memory consolidation during sleep. Nat Neurosci. 2016 Jul;19(7):959–64.
21. Packer AM, Roska B, Häusser M. Targeting neurons and photons for optogenetics. Nat Neurosci. 2013 Jul;16(7):805–15.
22. Leinenga G, Götz J. Scanning ultrasound removes amyloid-β and restores memory in an Alzheimer's disease mouse model. Sci Transl Med. 2015 Mar 11;7(278):278ra33.
23. Zhu H, Pleil KE, Urban DJ, Moy SS, Kash TL, Roth BL. Chemogenetic inactivation of ventral hippocampal glutamatergic neurons disrupts consolidation of contextual fear memory. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol. 2014 Jul;39(8):1880–92.
24. Wang J, Tanila H, Puoliväli J, Kadish I, van Groen T. Gender differences in the amount and deposition of amyloidbeta in APPswe and PS1 double transgenic mice. Neurobiol Dis. 2003
25. Yue M, Hanna A, Wilson J, Roder H, Janus C. Sex difference in pathology and memory decline in rTg4510 mouse model of tauopathy. Neurobiol Aging. 2011

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	Circuit manipulations in behaving, head-fixed mice
2	Circuit manipulation in freely moving mice

5.

Appendix
Description animal procedures

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 1	Type of animal procedure Circuit manipulations in behaving, head-fixed mice

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The aim of this experiment will be to investigate brain dynamics in the brain areas linked to amyloid related hyperactivity and interacting areas, during memory and sensory dependent behaviors.

Experiments with awake, head-fixed mice are an important complementary method to freely moving and anesthetized preparations. Whereas freely moving animals allow for more complex naturalistic behaviors, head-fixed experiments have several important advantages:

By fixing the animal's position within an experimental setup and restricting it in its behavioral repertoire (while still allowing to perform the behaviors of interest), precise description of the animal's behavior and location is facilitated enormously. In addition, exact sensory stimulation is made possible. This stimulation could for example be real objects that are presented to the animal, or virtual stimuli such as images and scenes on a screen, or controlled stimulation of the rodent's whiskers. To leverage the natural behavior of rodents to run around while interacting with their environment, we will usually combine head-fixation with a mechanism that allows the rodent to perform walking and running movements while being fixated. These mechanisms can, for example, be (spherical) treadmills, wheels, or air suspended platforms that the rodent can move to create the illusion of running. In combination with a virtual reality setup, this additionally allows to create experiments in which the rodents can interact with huge, practically infinitely large environments, making it possible to assess complex forms of memory guided behavior.
A second advantage of head-fixed animals is the possibility to use more complex neural recording techniques – most imaging techniques, for example, require the animal to be still while an image is acquired. Semi-acute electrophysiological recordings, in which the recording electrodes are inserted into the brain in each session significantly decrease the time and complexity of preparatory surgeries (thus reducing the animal's discomfort and increasing success rate) while giving the freedom to easily adjust electrode position between experimental sessions and thereby increasing the yield of neural measurements.

Ultimately the goal of using these state of the art recording techniques in combination with methods to manipulate neural activity during memory dependent tasks is to infer causal relationships between neural activity and memory performance.

.

The primary outcome measurements of these experiments are:
1. Measurements of behavioral performance of head-fixed mice during various memory and sensory related tasks including test of sensory memories, spatial memories and object memories (in a virtual reality environment). For instance, memory testing will be performed in a head-fixed,

virtual reality version of the Morris Water Maze, where APP-PS1 are known to be impaired and, pharmacological treatments are known to have a partial rescue effect. Our pilot studies show that mice can learn maze tasks quite effectively in this kind of setup. Parameters such as percent correct, latency to response, confusion matrix (which responses are measured with which stimuli) will be measured. Animal motion will be measured by sensing the movement of the track ball, treadmill or wheel that supports the animal. Additional bodily parameters such as ECG, or pupil diameter, gaze direction (by infrared camera) may be measured in non-invasive fashions.

2. Electrophysiological recordings of local network (single cell & neural ensemble) and global network level (local field potentials, EEG) activity in a network of memory related brain structures such as the hippocampus, entorhinal cortex or sensory areas such as barrel cortex and connected thalamic areas in awake head-fixed rodents that perform memory related tasks, to obtain fine time resolution measures of memory-related brain activity

3. Calcium and voltage-sensitive dye imaging measurements with two-photon microscopes and mini-cameras to obtain high throughput (hundreds of neurons simultaneously monitored), medium-time (10-100 ms) resolution monitoring of neural activity in a network of memory related structures in awake head-fixed mice that perform memory related tasks.

4. Optogenetic, pharamcogenetical and pharmacological perturbation of neural functioning, in combination with imaging or recording of neural activity in the same or related brain structures and/or analysis of behavioral changes during the perturbation. This could for example be optogenetic stimulation of neurons, or pharmacological blockade of a specific neurotransmitter in mice that perform a memory guided sensory discrimination task.

5. Use of electrophysiological and optical imaging techniques as well as optogenetic/ electrical /ultrasound manipulations in a network of memory related structures in anesthetized animals, for characterization of basic network properties, independent of behavior

6. *Ex-vivo* Histological measures of gene expression, concentrations of relevant chemicals (e.g. neuromodulators, neurotransmitters), neuroanatomy, validation of implant placement.

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

Animals will be accommodated to the housing facilities following the usual procedures in our facility. To motivate the natural running behavior of rodents, which is essential for experiments in which running is required, the animals will be provided with additional cage enrichment, for example in the form of running wheels, toys, or objects to explore, unless this is deemed unnecessary or detrimental to animal welfare, based on observation of subject behavior.

Preparation and Surgery:
For behavioral experiments, at the beginning of each experiment the animals have to undergo a surgery including different steps depending on the group

- head-post (for head restraint) implant
- Implantation of optic fibers
- the preparation of craniotomies or the implantation of electrodes to ensure target brain areas are accessible for later recording sessions
- implantations of cranial windows
- Implantation of microendoscopic lenses
- the injection of viral vectors for calcium/voltage sensitive dye and optogenetic / pharmacogenetical studies

Depending on the specific projects and complexity of the procedures, these preparations can be either performed in one or more (max. 3) separate stereotactic surgeries. A second surgery is required as some steps require an interval of weeks between them. Injections of viral vectors, for example, has to be performed in advance to allow sufficient time for viral expression. A 2-10 weeks incubation period, depending on the transfected gene and the target structure. This can be combined with the head-post implantation but not with other implantation procedures, to reduce the risk of infection in craniotomies. In the case of microscopy techniques such as mini-camera imaging, these require sufficient expression of the injected viral construct and the availability of fluorescent signals at surgery for implantable components (lenses, cranial windows) to be correctly positioned. This needs to be done in two successive steps, to ensure correct positioning of the lens and the microscope
In general, we will strive to minimize the number of surgeries necessary.
Animals that undergo viral injections need to remain in quarantine in independently ventilated cages for a 1-3 week periods, according to GGO regulations.

Manipulations
Animals are subjected to pharmacological, optogenetical, pharmacogenetical or electrical manipulation to restore and/or prevent abnormal neuronal activity. In the goal two the aim is to characterize the mechanism of action of different treatments for AD related hyperactivity. The animals will be treated with acute intraperitoneal injection of a low dose of clonazepam (0.05 mg/kg), or with antiepileptic drug, levetiracetam, acutely 200 mg/kg i.p., or chronically 75 mg/kg daily administered by miniosmotic pumps (e.g. Alzet miniosmotic pumps http://www.alzet.com/) for 28 days. The dosing is based on previous animal and human experiments for controlling hyperactivity in memory decline (7,8,19). Alternatively, intracortical bipolar stimulation is achieved by stimulating the deep part of the electrode in the cortex (20). Optogenetical manipulation is achieved by injecting viral vectors carrying a gene construct for light sensitive ion channel. Additionally, animals are equipped with small chronic optic cable during electrode implantation (21). Ultrasound stimulation of brain areas will be achieved by an integrated focused ultrasound system was used (22). Pharmacogenetical manipulation will be based on A DREADD, designer receptor, which will be expressed in neurons. Since the HM4Di has no natural ligands and respond exclusively to a synthetic small molecule, clozapine N-oxide (CNO), administration of CNO provides us a temporally and spatially specific method to control hyperactive (23).

Training/ Recordings
Behavioral experiments: All tasks used are of an appetitive nature. Training is done gradually and with positive reinforcement to support the animals' habituation to the training/recording setup and task.

Initially, animals are habituated to restraint and to head fixation, and then trained in the specific task of interest. Animals might be mildly food or water restricted to motivate behavioral performance of the animal with food or fluid. All food restricted animals will be monitored closely to ensure sufficient food intake. To protect neural implants or to ensure equal food availability and sufficient food intake, animals with implants will need to be housed singularly.

Training continues between 1 day and several weeks (max. 12 weeks with an animal) for more complex tasks but generally until a certain behavioral criterion is reached (e.g. 70% trials correct in 1 session). Sessions usually last between 30 minutes and 2-3 hours per day, where care is taken to only continue the session while the animal is accepting the head fixation.

During semi-acute and acute electrophysiological recording sessions, electrodes are inserted into the brain structures of interest and recordings are performed. Due to the lack of sub-dural pain receptors, mice usually take very little notice of this procedure even without anesthesia and analgesia.
Optical imaging can usually be done by placing the imaging microscope or mini camera over the previously prepared imaging area and don't require additional invasive procedures.
Recording session, electrical and optical, will take place daily, either after behavioral criterion is reached, or during training, depending on the task.

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

Animals will be accommodated to the housing facilities following the usual procedures in our facility. To motivate the natural running behavior of rodents, which is essential for experiments in which running is required, the animals will be provided with additional cage enrichment, for example in the form of running wheels, toys, or objects to explore, unless this is deemed unnecessary or detrimental to animal welfare, based on observation of subject behavior.

Preparation and Surgery:
For behavioral experiments, at the beginning of each experiment the animals have to undergo a surgery including different steps depending on the group

- head-post (for head restraint) implant
- Implantation of optic fibers
- the preparation of craniotomies or the implantation of electrodes to ensure target brain areas are accessible for later recording sessions
- implantations of cranial windows
- Implantation of microendoscopic lenses
- the injection of viral vectors for calcium/voltage sensitive dye and optogenetic / pharmacogenetical studies

Under certain circumstances it will be necessary to put the animal under anesthesia to repair or improve the efficiency of the implant.
Depending on the specific projects and complexity of the procedures, these preparations can be either performed in one or more (max. 3 e.g. head post implantation + injection of viral vectors + implantation of optic fibers) separate stereotactic surgeries. A second surgery is required as some steps require an interval of weeks between them. Injections of viral vectors, for example, has to be performed in advance to allow sufficient time for viral expression. A 2-10 weeks incubation period, depending on the transfected gene and the target structure. This can be combined with the head-

post implantation but not with other implantation procedures, to reduce the risk of infection in craniotomies. In the case of microscopy techniques such as mini-camera imaging, these require sufficient expression of the injected viral construct and the availability of fluorescent signals at surgery for implantable components (lenses, cranial windows) to be correctly positioned. This needs to be done in two successive steps, to ensure correct positioning of the lens and the microscope
In general, we will strive to minimize the number of surgeries necessary.
Animals that undergo viral injections need to remain in quarantine in independently ventilated cages for a 1-3 week periods, according to GGO regulations.

Manipulations
The animals will be treated with acute intraperitoneal injection of a low dose of clonazepam (0.05 mg/kg) or with antiepileptic drug, levetiracetam, acutely 200 mg/kg i.p., or chronically 75 mg/kg daily administered by miniosmotic pumps (e.g. Alzet miniosmotic pumps http://www.alzet.com/) for 28 days. The dosing is based on previous animal and human experiments for controlling hyperactivity in memory decline (29,30,42). Intracortical bipolar stimulation is achieved by stimulating the deep part of the electrode in the cortex (11). Optogenetical manipulation is achieved by injecting once viral vectors carrying a gene construct for light sensitive ion channel (12). Additionally, animals are equipped with small chronic optic cable during electrode implantation (13). Pharmacogenetical manipulation will be based on A DREADD, designer receptor, which will be expressed in neurons. Since the HM4Di has no natural ligands and respond exclusively to a synthetic small molecule, clozapine N-oxide (CNO), administration once or several times of CNO provides us a temporally and spatially specific method to control hyperactive (13).

Training/ Recordings
Behavioral experiments: All tasks used are of an appetitive nature. Training is done gradually and with positive reinforcement to support the animals' habituation to the training/recording setup and task.
Initially, animals are habituated to restraint and to head fixation, and then trained in the specific task of interest. Animals might be mildly food or water restricted to motivate behavioral performance by rewarding the animal with food or fluid reward. All food restricted animals will be monitored closely to ensure sufficient food intake. To protect neural implants or to ensure equal food availability and sufficient food intake, animals with implants will need to be housed singularly. We have tried it before with rats and mice. If rats start to argue the implant is among the first thing they bite. We saw it and there was pieces bit off from the head implant. Also with mice we have had the same issues. Since it is impossible to prevent the fighting and nibbling it is too risky to keep 2 animals in the same cage. Even one bite can destroy (reference or ground) the whole recording system and consequently I need to sacrifice the animal.

Training continues between 1 day and several weeks (max. 12 weeks with an animal) for more complex tasks but generally until a certain behavioral criterion is reached (e.g. 70% trials correct in 1 session). Sessions usually last between 30 minutes and 2-3 hours per day, where care is taken to only continue the session while the animal is accepting the head fixation.

During semi-acute and acute electrophysiological recording sessions, electrodes are inserted into the brain structures of interest and recordings are performed. Due to the lack of sub-dural pain receptors, mice usually take very little notice of this procedure even without anesthesia and analgesia.
Optical imaging can usually be done by placing the imaging microscope or mini camera over the previously prepared imaging area and don't require additional invasive procedures.
Recording session, electrical and optical, will take place daily, either after behavioral criterion is reached, or during training, depending on the task.

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

For each experimental group, the number of animals will be assessed by power analysis. Power analysis will take into account the high yield in terms of recorded cells of our methodologies. For each measure, the appropriate N number will be selected, which may be number of animals, or number of recorded neurons, or number of electrodes, etc. Because these are novel procedures, some pilot experiments will be needed to assess the effect sizes before a proper analysis can be performed. When possible we will use data from similar experiments in the literature for an initial guess of the effect sizes. Number of animals will be increased to allow for dropout due to non-functioning implants, problems at surgery, or other causes of experimental failure. The maximum group size for each group, based on our experience, will be 35 animals (15 wild types + 20 transgenic).

By using combinations state-of-the-art high-yield recording techniques, we constantly increase the number of neurons recorded per animal, and hence reduce the total amount of animals needed per group. In all cases, we expect a dropout rate for these complications of about 30% for both transgenic and wild types. In addition, we expect additional maximum 20 % dropout rate for only to transgenic animals because of the plausible epileptic activity (see the Coherence section).

B. The animals

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

The hypotheses will be tested with male mice carrying human APPswe and PS1dE9 (APdE9) mutations which develop amyloid plaques beginning at 3-4 months of age without memory dysfunction. These animals show memory impairment in memory tasks around 10-12 months of age when these animals also suffer from severe amyloid plaque burden (9–11). These mice show increase in epileptic seizures between 3 and 4.5 months of age reaching eventually a prevalence of 60% of the population (3). At this age, as we have shown, mice also display cortical and-thalamic hyperactivity (4). Therefore, studying the young animals may lead to the detection of early EEG biomarkers for early stages of AD, and studies with older animals to the characterization of the pathology of the cognitive loss.

For breeding 100 transgenic male are used.

Group size will be determined by power analysis as explained above. Each group will include transgenic animals and wild type litter mates as control animals as needed for the specific question. The maximum group size for each group, based on our experience, will be 35 animals per group (20 transgenic and 15 wild type littermates as determined by power analysis). There will be 3 groups with both early and late stage mice, so the total amount of animals will be 210 animals. The detailed description of groups is provided in the figure 1 and in the table 2 (groups 9-11).

Life stages

The experiments will be performed in mice starting from 4-6 weeks until up to 18 months.

Table 2. Description of manipulations and procedures, and the discomfort level in different experimental groups. The head fixed animals are marked as the groups 9-11.

Groups #	Experiment	Manipulations and procedures				Discomfort	Number of animals	
Number of animals total 210 **Out of which** • **Moderate discomfort: 140** • **Severe discomfort: 70**		Surgical procedures If 2 -> SEVERE If 1 -> MODERATE						
		Virus injection	Elect. implantation	Manipulation of neural activity	Behavioral test		Young	Old
9	Electrophysiological characterization of neuronal activity and pharmacological manipulation	NO	YES	YES	YES	Cumulative: MODERATE	35	35
10	Electrophysiological characterization of neuronal activity and optogenetical/pharmacogenetical manipulation	YES	YES	YES	YES	Cumulative: SEVERE	35	35
11	Electrophysiological characterization of neuronal activity and electrical / ultrasound manipulation	NO	YES	YES	YES	Cumulative: MODERATE	35	35

Species	Origin	Maximum number of animals	Life stage

Mouse wt	own breeding	45	early stage 2-10 m
mouse tg	own breeding	60	early state 2-10 m
mouse tg	own breeding	60	late stage 11-18 m
mouse wt	own breeding	45	late stage 11-18 m
mouse tg	own breeding	100	for breeding until 14 months

C. Re-use

Will the animals be re-used?

[] No, continue with question D.
[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that were used in one memory task may, when in good health conditions and when experimentally appropriate, be re-tested in a another memory task, helping reducing the number of animals used in total

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

[] No
[X] 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 At the moment, there are no alternative techniques capable of providing data about the relationship between behavior, learning, genetic modifications, and neural code. While our group has also expertise in computational modeling of neural systems, which are part of our research approach, for the questions that we target here there are too many missing data about biological neural network behavior for a purely theoretical approach.
We use rodents because the procedures for electrophysiological recordings, optogenetic stimulation and calcium/voltage sensitive dye imaging have been best fine-tuned in this species and to avoid use primates. Mice can perform complex memory tasks that cannot be performed by lower species while most of the brain areas know to be involved in these tasks have homologous counterparts in humans.

Reduction. It is to be noted that the high data-yield of our techniques permits a significant reduction in the number of animals needed to attain statistical significance in neurophysiological measures. Collecting the same amount of data with more traditional methodologies may require many times more animals.
The number of animals will be determined by power analyses which will take the expected experimental yield of our techniques in terms of recorded neurons into account.

Refinement The electrophysiological and behavioral techniques employed are established in the lab. Surgeries will employ advanced pain control (see below) and sterility techniques. Implantable devices are engineered (in many cases in-house) to obtain the lowest weight, smallest bulk, and maximum comfort for the animals, and are comparable with the state-of-the-art in the field in this respect. We will continue to refine our surgical techniques and devices, in order to minimize animal discomfort and adverse health consequences, as we have done in the past.

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

All animals will receive intensive pre and postsurgical care including anti-analgesic treatment, daily check of their health status and environmental enrichment. Anesthesia will be administered under monitoring of vital parameters (spO2, heart rate).
Animals will gradually be habituated to the head fixed setup e.g.according to protocols developed by our collaborator.
During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Habituation to head-fixation will be done gradually and with positive reinforcement in a low-light environment. In our experience mice usually adapt quickly to this treatment and have been shown exhibit normal foraging, food consumption, grooming and sleeping behavior.

We use transgenic animals and genetically engineered viral vectors (of low-risk class) according to GGO national regulations, for prevention of human and environmental contamination.

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.

Not applicable

Accommodation and care

F. Accommodation and care

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

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

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

[X] No > Continue with question H.
[] Yes > Describe this establishment.

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

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

Surgeries will require anesthesia and pain relief
Rodents will be anesthetized with injection drugs, e.g. ketamine/ medetomidine or urethane (for non-recovery procedures) or gas (e.g. isoflurane) during surgeries, according to state-of-the-art accepted protocols, developed in collaboration with the IvD and animal facility personnel. Atropine will be administered to support respiratory function during surgeries. Analgesia will be guaranteed with carprofen, meloxicam or other approved agents during and after surgeries.

I. Other aspects compromising the welfare of the animals

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

Breeding: Breeding might cause mild discomfort for breeding animals

Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

Handling stress: animals will be gradually habituated to handling.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (for mice: never below 90% body weight, restriction only in the hours right before the experiment).
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APdE9 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint. In most cases the risk for discomfort is moderate at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

Explain why these effects may emerge.

Breeding: Breeding might cause mild discomfort for breeding animals

Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

Handling stress: animals will be gradually habituated to handling.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (for mice: never below 90% body weight, restriction only in the hours right before the experiment).
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APdE9 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint. In most cases the risk for discomfort is moderate at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

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

Breeding: Breeding might cause mild discomfort for breeding animals

Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

Handling stress: animals will be gradually habituated to handling.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (for mice: never below 90% body weight, restriction only in the hours right before the experiment).
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APdE9 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint. In most cases

the risk for discomfort is moderate at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

J. Humane endpoints

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

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

The following general humane endpoints will be applied:

1) When recovery after surgery is insufficient. In our experience, animals may lose some weight in the days immediately following the surgery. However, it will be considered if the animal shows permanent weight loss (more than 20% of the weight after the surgery for more than 7 days). In the case of juvenile animals, a lack of weight gain compatible with the animal life stage will be also considering a sign of impaired well-being.
2) When there are post-surgical complications, e.g. infection in the incision.
3) If pathogenesis without hope of recovery is determined. Visible signs of pathogenesis will be monitored such as infection, and condition of the fur. The following will be considered as signs of unhealthy state of the animal:
a. Aberrant behavior (e.g. freezing)
b. Shock
c. Dehydration
d. Weight loss
e. Nose and Mouth discharge
f. Bleeding
g. Fits/Seizures as described previously
h. Diarrhea

Should these unhealthy symptoms appear we will contact the vet to examine possible causes and monitor the animal closely to observe any improvement or decline in the condition of the animal. The vet will be consulted to judge the likelihood of recovery given the nature of the symptoms and their development. If recovery is judged unlikely or to involve prolonged suffering, then the humane endpoint will be reached.

4) When electrophysiological readings show that the head-plate is no longer firmly in place. This will be shown by a disruption of the normally stable background noise and an inability to stably record cells for a number of consecutive sessions. A recovery operation is then no longer possible.

5) When the animal is not learning the behavioral task after repeated attempts. Even if there is no worse discomfort conditions than foreseen, the experiment will be terminated, to prevent pointless manipulation

Indicate the likely incidence.

1: <10%
2: <5%
3: <5%
4: <5%
5: <5%

total < 30%

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).

Breeding: Mild, breeding animals

Handling stress: mild groups 9-11

Injection: mild, groups 10-11

Surgery and recovery: moderate groups 9 and 11, severe 10

Cumulative discomfort: moderate groups 9 and 11, severe 10

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

The animal will be killed as part of the procedure to allow us to perform perfusion and histological analysis.

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

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

Appendix
Description animal procedures

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure Circuit manipulation in freely moving mice

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The aim of these experiments is to investigate dynamics of memory within and between (interacting) relevant brain areas such as the hippocampus and neocortex in freely moving mice during (memory-) behavioral tasks. Although the use of head-fixed animals is motivated by several benefits as explained in [appendix 1], in terms of the relative ease by which complex experimental configuration may be achieved, the use of freely moving animals has the significant advantage of testing animals in a realistic or natural environment that cannot be achieved in a head-fixed setting, such as testing memory that involves direct exploration of an object or certain tasks involving spatial navigation, which heavily depends on vestibular inputs. For example it is known that neural correlates of spatial location in the hippocampus are quite different in a head-restrained, virtual reality condition compared to the freely moving situation.

In these series of experiments, we will combine state-of-the-art techniques to measures neural correlates of memory-dependent behavior to provide novel insight that will further our understanding in basic memory processes but also significantly contribute to our understanding of complex diseases and neuropsychiatric disorders where a memory deficit is a key feature.

The primary outcome measurements of these experiments are:
1. Behavioral readouts of freely moving animals in memory-related tasks for instance delayed T-test or object recognition task in which we will study various stages of memory, namely encoding, retention, consolidation and retrieval and how they are effected by progressive amyloid pathology. These tasks will involve spatial memory, recognition memory and working memory. The readout will involve e.g. percentage of trials correct, latency to response and time spent exploring.
2. Electrophysiological recordings of local and global level neural activity in memory-relevant brain for instance the hippocampus, entorhinal cortex, barrel cortex and thalamus areas to study neural correlates on a microcircuit level and the network level.
3. In vivo calcium activity measurements of memory-related areas to study individual cell activity during memory tasks with high spatial and temporal resolution
4. Optogenetic, pharmacogenetical or electrical stimulation manipulation of brain activity to study how increasing or suppressing activity in one brain area affects the behavior of the animal and how this affects activity in interacting brain areas. Stimulation may be delivered at arbitrary times, or triggered by the detection of specific patterns in neural activity
5. Measurement of the behavioral and neurophysiological effects of pharmacological manipulations, local or systemic
6. Ex-vivo Histological measures of gene expression, concentrations of relevant chemicals (e.g. neuromodulators, neurotransmitters), neuroanatomy, validation of implant placement.

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

The aim of the experiments is to investigate memory dynamics in vivo by using freely moving mice to manipulate and record or image brain activity in relevant brain areas during memory-relevant tasks. Behavioral procedures will depend on the state of the literature in this rapidly evolving field at the moment of the beginning of each experiment, and on the data we collect in previous experiment, but examples of envisaged experiments are:

- the characterization of spatial responses in the hippocampal formation and how they respond to behavioral as well as pharmacological, pharmacogenetical and optogenetic manipulations
- Recognition (e.g. of objects) memory tasks

In all cases, we will measure spontaneous ongoing behavior, or will reward the desired responses with an appetitive reward. In this procedure, no aversive conditioning (e.g. foot shocks) are foreseen.

Preparation and surgery

Surgeries: stereotaxic surgery will be required for brain implants, used to record and manipulate brain activity.
We will implant the following types of devices

1) electrode arrays/electrode probes
2) optic fibers for optogenetic stimulation
3) miniature lenses for microendoscope imaging

Depending on the specific projects and complexity of the procedures, these preparations can be either performed in one or more (max. 3) separate stereotactic surgeries. A second surgery is required as some steps require an interval of weeks between them. Injections of viral vectors (used in all groups), for example, has to be performed in advance to allow sufficient time for viral expression. A 2-10 weeks incubation period, depending on the transfected gene and the target structure. This cannot be combined with other implantation procedures, to reduce the risk of infection in craniotomies, if too long a time elapses between the implantation and the
In the case of microscopy techniques such as mini-camera imaging, these require sufficient expression of the injected viral construct and the availability of fluorescent signals at surgery for implantable components (lenses, cranial windows) to be correctly positioned. This needs to be done in two successive steps, to ensure correct positioning of the lens and the microscope. These groups will underlie three surgeries

Under certain circumstances it will be necessary to put the animal under anesthesia to repair or improve the efficiency of the implant.

To protect neural implants or to ensure equal food availability and sufficient food intake, animals with implants will need to be housed singularly.

Manipulations
Animals are subjected to pharmacological, optogenetical, pharmacogenetical or electrical manipulation to restore and/or prevent abnormal neuronal activity. In the goal two the aim is to characterize the mechanism of action of different treatments for AD related hyperactivity. The animals will be treated with acute intraperitoneal injection of a low dose of clonazepam (0.05 mg/kg), or with antiepileptic drug, levetiracetam, acutely 200 mg/kg i.p., or chronically 75 mg/kg daily administered by miniosmotic pumps (e.g. Alzet miniosmotic pumps http://www.alzet.com/) for 28 days. The dosing is based on previous animal and human experiments for controlling hyperactivity in memory decline (7,8,19). Alternatively, intracortical bipolar stimulation is achieved by stimulating the deep part of the electrode in the cortex (20). Optogenetical manipulation is achieved by injecting viral vectors carrying a gene construct for light sensitive ion channel. Additionally, animals are equipped with small chronic optic cable during electrode implantation (21). Ultrasound stimulation of brain areas will be achieved by an integrated focused ultrasound system was used (22). Pharmacogenetical manipulation will be based on A DREADD, designer receptor, which will be expressed in neurons. Since the HM4Di has no natural ligands and respond exclusively to a synthetic small molecule, clozapine N-oxide (CNO), administration of CNO provides us a temporally and spatially specific method to control hyperactive (23).

Behavioral training
Animals will be trained in several memory- or sensory-related tasks. Some of these tasks will require food or water restriction for optimal performance of the animal. Training of the animals will be conducted 5-6 days per week and depending on the task will require to be trained until the animal has reached criterion (>80% trials correct).
Optical or electrical manipulations will be performed during various phases of the memory task, such as encoding, retention, consolidation and retrieval. The readout will consist of the behavioral performance, electrophysiological recordings and/or imaging of cell activity.

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

For each experimental group, the number of animals will be assessed by power analysis. Power analysis will take into account the high yield in terms of recorded cells of our methodologies. For each measure, the appropriate N number will be selected, which may be number of animals, or number of recorded neurons, or number of electrodes, etc. Because these are novel procedures, some pilot experiments will be needed to assess the effect sizes before a proper analysis can be performed. When possible we will use data from similar experiments in the literature for an initial guess of the effect sizes. Number of animals will be increased to allow for dropout due to non-functioning implants, problems at surgery, or other causes of experimental failure. The maximum group size for each group, based on our experience, will be 35 animals (15 wild types + 20 transgenic).

By using combinations state-of-the-art high-yield recording techniques, we constantly increase the number of neurons recorded per animal, and hence reduce the total amount of animals needed per group. In all cases, we expect a dropout rate for these complications of about 30% for both transgenic and wild types. In addition, we expect additional maximum 20 % dropout rate for transgenic animals because of the plausible epileptic activity (see the Coherence section).

B. The animals

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

The hypotheses will be tested with male mice carrying human APPswe and PS1dE9 (APdE9) mutations which develop amyloid plaques beginning at 3-4 months of age without memory dysfunction. These animals show memory impairment in memory tasks around 10-12 months of age when these animals also suffer from severe amyloid plaque burden (9–11). These mice show increase in epileptic seizures between 3 and 4.5 months of age reaching eventually a prevalence of 60% of the population (3). At this age, as we have shown, mice also display cortical and-thalamic hyperactivity (4). Therefore, studying the young animals may lead to the detection of early EEG biomarkers for early stages of AD, and studies with older animals to the characterization of the pathology of the cognitive loss.

Group size will be determined by power analysis as explained above. Each group will include transgenic animals and wild type litter mates as control animals as needed for the specific question. The maximum group size for each group, based on our experience, will be 35 animals per group (20 transgenic and 15 wild type littermates as determined by power analysis). There will be 8 groups with both early and late stage mice, so the total amount of animals will be 560 animals.

For breeding 300 transgenic males are used.
The detailed description of groups is provided in the figure 1 and in the table 3 (groups 1-8).

Groups #	Experiment	Manipulations and procedures						Discomfort	Number of animals	
Number of animals total 560 **Out of which** • **Moderate discomfort: 280** • **Severe discomfort: 280**		Surgical procedures If more than 1 -> SEVERE If 1 -> MODERATE				Manipulation of neural activity	Behavioral test		Young	Old
		Virus injection	Electrode implantation	Lens impalntation Lens surgery	Lens base surgery					
1-4	Electrophysiological characterization of neuronal activity and pharmacological / electrical manipulation	NO	YES	NO	NO	YES	YES	Cumulative: MODERATE	4x35 =140	4x35 =140
5	Electrophysiological characterization of neuronal activity and optogenetical or pharmacogenetical manipulation	YES	YES	NO	NO	YES	YES	Cumulative: SEVERE	35	35
6-8	Calcium imaging of neuronal activity and pahramacological or optogenetical or pharmacogenetical manipulation (Implantation of calcium imaging lens needs 2 operations!, 3 surgical procedures in these groups)	YES	NO	YES	YES	YES	YES	Cumulative: SEVERE	3x35 =105	3x35 =105

Life stages
The experiments will be performed in mice starting from 4-6 weeks until up to 18 months.

Species	Origin	Maximum number of animals	Life stage
mouse wt	own breeding	120	early stage 2-10 months
mouse tg	own breeding	160	late stage 11-18
mouse tg	own breeding	160	early stage 2-10 months
mouse wt	own breeding	120	late stage 11-18
mouse tg	own breeding	300	for breeding till 14 months

C. Re-use

Will the animals be re-used?

[] No, continue with question D.
[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that were used in one memory task may, when in good health conditions and when experimentally appropriate, be re-used for a different memory task, helping reducing the number of animals used in total. In no case will an animal be measured for more than 2 months, or be in an experiment after 1 year of age. Animals experiencing discomfort beyond moderate will not be re-used.

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

[] No
[X] 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 At the moment, there are no alternative techniques capable of providing data about the relationship between behavior, learning, genetic modifications, and neural code. While our group has also expertise in computational modeling of neural systems, which are part of our research approach, for the questions that we target here there are too many missing data about biological neural network behavior for a purely theoretical approach.
We use rodents because the procedures for electrophysiological recordings, optogenetic stimulation and calcium/voltage sensitive dye imaging have

been best fine-tuned in this species and to avoid use primates. Mice can perform complex memory tasks that cannot be performed by lower species while most of the brain areas know to be involved in these tasks have homologous counterparts in humans.

Reduction. It is to be noted that the high data-yield of our techniques permits a significant reduction in the number of animals needed to attain statistical significance in neurophysiological measures. Collecting the same amount of data with more traditional methodologies may require many times more animals.
The number of animals will be determined by power analyses which will take the expected experimental yield of our techniques in terms of recorded neurons into account.

Refinement The electrophysiological and behavioral techniques employed are established in the lab. Surgeries will employ advanced pain control (see below) and sterility techniques. Implantable devices are engineered (in many cases in-house) to obtain the lowest weight, smallest bulk, and maximum comfort for the animals, and are comparable with the state-of-the-art in the field in this respect. We will continue to refine our surgical techniques and devices, in order to minimize animal discomfort and adverse health consequences, as we have done in the past.

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

All animals will receive intensive pre and postsurgical care including anti-analgesic treatment, daily check of their health status and environmental enrichment.
Anesthesia will be administered under monitoring of vital parameters (spO2, heart rate).
During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. However, daily periods of social interactions with former littermates can usually be arranged after training.
During this period, animals will be checked daily for their health status and will receive additional food if required.
Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

We use transgenic animals and genetically engineered viral vectors (of low-risk class) according to GGO national regulations, for prevention of human and environmental contamination.

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.

Not applicable

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

After the surgery the animals are housed in individual cages because of veterinary concerns regarding animal well-being related to allow a short term recovery post-operatively and restrict a long term aggressive behaviour of cage mates towards animal with chronical implant.

G. Location where the animals procedures are performed

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

[X] No > Continue with question H.
[] Yes > Describe this establishment.

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

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

Surgeries will require anesthesia and pain relief.
Mice will be anesthetized with injection drugs, e.g. ketamine/ medetomidine or urethane (for non-recovery procedures) or gas (e.g. isoflurane) during surgeries, according to state-of-the-art accepted protocols, developed in collaboration with the IvD and animal facility personnel. Atropine will be administered to support respiratory function during surgeries. Analgesia will be guaranteed with carprofen, meloxicam or other approved agents during and after surgeries.

I. Other aspects compromising the welfare of the animals

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

Breeding: Breeding might cause mild discomfort for breeding animals
Handling stress: animals will be gradually habituated to handling.
Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (never below 90% body weight), restriction only in the hours right before the experiment.
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APP/PS1 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint.

In most cases the risk for discomfort is moderate (3) at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

Explain why these effects may emerge.

Breeding: Breeding might cause mild discomfort for breeding animals

Handling stress: animals will be gradually habituated to handling.
Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (never below 90% body weight), restriction only in the hours right before the experiment.
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APP/PS1 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint.
In most cases the risk for discomfort is moderate (3) at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

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

Breeding: Breeding might cause mild discomfort for breeding animals

Handling stress: animals will be gradually habituated to handling.
Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment.

Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (never below 90% body weight), restriction only in the hours right before the experiment.
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APP/PS1 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint.
In most cases the risk for discomfort is moderate (3) at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

J. Humane endpoints

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

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

1) When recovery after surgery is insufficient. In our experience, animals may lose some weight in the days immediately following the surgery. However, it will be considered if the animal shows permanent weight loss (more than 20% of the weight after the surgery for more than 7 days).
2) When there are post-surgical complications, e.g. infection in the incision.

3) If pathogenesis without hope of recovery is determined. Visible signs of pathogenesis will be monitored such as infection, and condition of the fur. The following will be considered as signs of unhealthy state of the animal:
Aberrant behavior (e.g. freezing)

Shock

Dehydration

Weight loss

Nose and Mouth discharge

Bleeding

Fits/ Seizures as described previously

Diarrhea

Should these unhealthy symptoms appear we will contact the vet to examine possible causes and monitor the animal closely to observe any improvement or decline in the condition of the animal. The vet will be consulted to judge the likelihood of recovery given the nature of the symptoms and their development. If recovery is judged unlikely or to involve prolonged suffering, then the humane endpoint will be reached.

4) When electrophysiological readings show that the head-plate is no longer firmly in place. This will be shown by a disruption of the normally stable background noise and an inability to stably record cells for a number of consecutive sessions. A recovery operation is then no longer possible.

5) when the animal is not learning the behavioral task after repeated attempts. Even if there is no worse discomfort conditions than foreseen, the experiment will be terminated, to prevent pointless manipulation

Indicate the likely incidence.

1: <10%
2: <5%
3: <5%

4: <5%
5: <5%

total < 30%

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).

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).
Breeding: Mild, breeding animals
Handling stress: mild, groups 1-8

Injection: mild, groups 1-8

Surgery and recovery: moderate groups 1-4, severe groups 5-8

Cumulative discomfort: moderate groups 1-4, severe groups 5-8

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

The animal will be killed as part of the procedure to allow us to perform perfusion and histological analysis.

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

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

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.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 1	Type of animal procedure Circuit manipulations in behaving, head-fixed mice

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The aim of this experiment will be to investigate brain dynamics in the brain areas linked to amyloid related hyperactivity and interacting areas, during memory and sensory dependent behaviors.

Experiments with awake, head-fixed mice are an important complementary method to freely moving and anesthetized preparations. Whereas freely moving animals allow for more complex naturalistic behaviors, head-fixed experiments have several important advantages:

By fixing the animal's position within an experimental setup and restricting it in its behavioral repertoire (while still allowing to perform the behaviors of interest), precise description of the animal's behavior and location is facilitated enormously. In addition, exact sensory stimulation is made possible. This stimulation could for example be real objects that are presented to the animal, or virtual stimuli such as images and scenes on a screen, or controlled stimulation of the rodent's whiskers. To leverage the natural behavior of rodents to run around while interacting with their environment, we will usually combine head-fixation with a mechanism that allows the rodent to perform walking and running movements while being fixated. These mechanisms can, for example, be (spherical) treadmills, wheels, or air suspended platforms that the rodent can move to create the illusion of running. In combination with a virtual reality setup, this additionally allows to create experiments in which the rodents can interact with huge, practically infinitely large environments, making it possible to assess complex forms of memory guided behavior.
A second advantage of head-fixed animals is the possibility to use more complex neural recording techniques – most imaging techniques, for example, require the animal to be still while an image is acquired. Semi-acute electrophysiological recordings, in which the recording electrodes are inserted into the brain in each session significantly decrease the time and complexity of preparatory surgeries (thus reducing the animal's discomfort and increasing success rate) while giving the freedom to easily adjust electrode position between experimental sessions and thereby increasing the yield of neural measurements.

Ultimately the goal of using these state of the art recording techniques in combination with methods to manipulate neural activity during memory dependent tasks is to infer causal relationships between neural activity and memory performance.

.

The primary outcome measurements of these experiments are:
1. Measurements of behavioral performance of head-fixed mice during various memory and sensory related tasks including test of sensory memories, spatial memories and object memories (in a virtual reality environment). For instance, memory testing will be performed in a head-fixed,

virtual reality version of the Morris Water Maze, where APP-PS1 are known to be impaired and, pharmacological treatments are known to have a partial rescue effect. Our pilot studies show that mice can learn maze tasks quite effectively in this kind of setup. Parameters such as percent correct, latency to response, confusion matrix (which responses are measured with which stimuli) will be measured. Animal motion will be measured by sensing the movement of the track ball, treadmill or wheel that supports the animal. Additional bodily parameters such as ECG, or pupil diameter, gaze direction (by infrared camera) may be measured in non-invasive fashions.

2. Electrophysiological recordings of local network (single cell & neural ensemble) and global network level (local field potentials, EEG) activity in a network of memory related brain structures such as the hippocampus, entorhinal cortex or sensory areas such as barrel cortex and connected thalamic areas in awake head-fixed rodents that perform memory related tasks, to obtain fine time resolution measures of memory-related brain activity

3. Calcium and voltage-sensitive dye imaging measurements with two-photon microscopes and mini-cameras to obtain high throughput (hundreds of neurons simultaneously monitored), medium-time (10-100 ms) resolution monitoring of neural activity in a network of memory related structures in awake head-fixed mice that perform memory related tasks.

4. Optogenetic, pharamcogenetical and pharmacological perturbation of neural functioning, in combination with imaging or recording of neural activity in the same or related brain structures and/or analysis of behavioral changes during the perturbation. This could for example be optogenetic stimulation of neurons, or pharmacological blockade of a specific neurotransmitter in mice that perform a memory guided sensory discrimination task.

5. Use of electrophysiological and optical imaging techniques as well as optogenetic/ electrical /ultrasound manipulations in a network of memory related structures in anesthetized animals, for characterization of basic network properties, independent of behavior

6. *Ex-vivo* Histological measures of gene expression, concentrations of relevant chemicals (e.g. neuromodulators, neurotransmitters), neuroanatomy, validation of implant placement.

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

Animals will be accommodated to the housing facilities following the usual procedures in our facility. To motivate the natural running behavior of rodents, which is essential for experiments in which running is required, the animals will be provided with additional cage enrichment, for example in the form of running wheels, toys, or objects to explore, unless this is deemed unnecessary or detrimental to animal welfare, based on observation of subject behavior.

Preparation and Surgery:
For behavioral experiments, at the beginning of each experiment the animals have to undergo a surgery including different steps depending on the group

- head-post (for head restraint) implant
- Implantation of optic fibers
- the preparation of craniotomies or the implantation of electrodes to ensure target brain areas are accessible for later recording sessions
- implantations of cranial windows
- Implantation of microendoscopic lenses
- the injection of viral vectors for calcium/voltage sensitive dye and optogenetic / pharmacogenetical studies

Depending on the specific projects and complexity of the procedures, these preparations can be either performed in one or more (max. 3) separate stereotactic surgeries. A second surgery is required as some steps require an interval of weeks between them. Injections of viral vectors, for example, has to be performed in advance to allow sufficient time for viral expression. A 2-10 weeks incubation period, depending on the transfected gene and the target structure. This can be combined with the head-post implantation but not with other implantation procedures, to reduce the risk of infection in craniotomies. In the case of microscopy techniques such as mini-camera imaging, these require sufficient expression of the injected viral construct and the availability of fluorescent signals at surgery for implantable components (lenses, cranial windows) to be correctly positioned. This needs to be done in two successive steps, to ensure correct positioning of the lens and the microscope
In general, we will strive to minimize the number of surgeries necessary.
Animals that undergo viral injections need to remain in quarantine in independently ventilated cages for a 1-3 week periods, according to GGO regulations.

Manipulations
Animals are subjected to pharmacological, optogenetical, pharmacogenetical or electrical manipulation to restore and/or prevent abnormal neuronal activity. In the goal two the aim is to characterize the mechanism of action of different treatments for AD related hyperactivity. The animals will be treated with acute intraperitoneal injection of a low dose of clonazepam (0.05 mg/kg), or with antiepileptic drug, levetiracetam, acutely 200 mg/kg i.p., or chronically 75 mg/kg daily administered by miniosmotic pumps (e.g. Alzet miniosmotic pumps http://www.alzet.com/) for 28 days. The dosing is based on previous animal and human experiments for controlling hyperactivity in memory decline (7,8,19). Alternatively, intracortical bipolar stimulation is achieved by stimulating the deep part of the electrode in the cortex (20). Optogenetical manipulation is achieved by injecting viral vectors carrying a gene construct for light sensitive ion channel. Additionally, animals are equipped with small chronic optic cable during electrode implantation (21). Ultrasound stimulation of brain areas will be achieved by an integrated focused ultrasound system was used (22). Pharmacogenetical manipulation will be based on A DREADD, designer receptor, which will be expressed in neurons. Since the HM4Di has no natural ligands and respond exclusively to a synthetic small molecule, clozapine N-oxide (CNO), administration of CNO provides us a temporally and spatially specific method to control hyperactive (23).

Training/ Recordings
Behavioral experiments: All tasks used are of an appetitive nature. Training is done gradually and with positive reinforcement to support the animals' habituation to the training/recording setup and task.

Initially, animals are habituated to restraint and to head fixation, and then trained in the specific task of interest. Animals might be mildly food or water restricted to motivate behavioral performance of the animal with food or fluid. All food restricted animals will be monitored closely to ensure sufficient food intake. To protect neural implants or to ensure equal food availability and sufficient food intake, animals with implants will need to be housed singularly.

Training continues between 1 day and several weeks (max. 12 weeks with an animal) for more complex tasks but generally until a certain behavioral criterion is reached (e.g. 70% trials correct in 1 session). Sessions usually last between 30 minutes and 2-3 hours per day, where care is taken to only continue the session while the animal is accepting the head fixation.

During semi-acute and acute electrophysiological recording sessions, electrodes are inserted into the brain structures of interest and recordings are performed. Due to the lack of sub-dural pain receptors, mice usually take very little notice of this procedure even without anesthesia and analgesia.
Optical imaging can usually be done by placing the imaging microscope or mini camera over the previously prepared imaging area and don't require additional invasive procedures.
Recording session, electrical and optical, will take place daily, either after behavioral criterion is reached, or during training, depending on the task.

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

Animals will be accommodated to the housing facilities following the usual procedures in our facility. To motivate the natural running behavior of rodents, which is essential for experiments in which running is required, the animals will be provided with additional cage enrichment, for example in the form of running wheels, toys, or objects to explore, unless this is deemed unnecessary or detrimental to animal welfare, based on observation of subject behavior.

Preparation and Surgery:
For behavioral experiments, at the beginning of each experiment the animals have to undergo a surgery including different steps depending on the group

- head-post (for head restraint) implant
- Implantation of optic fibers
- the preparation of craniotomies or the implantation of electrodes to ensure target brain areas are accessible for later recording sessions
- implantations of cranial windows
- Implantation of microendoscopic lenses
- the injection of viral vectors for calcium/voltage sensitive dye and optogenetic / pharmacogenetical studies

Under certain circumstances it will be necessary to put the animal under anesthesia to repair or improve the efficiency of the implant.
Depending on the specific projects and complexity of the procedures, these preparations can be either performed in one or more (max. 3 e.g. head post implantation + injection of viral vectors + implantation of optic fibers) separate stereotactic surgeries. A second surgery is required as some steps require an interval of weeks between them. Injections of viral vectors, for example, has to be performed in advance to allow sufficient time for viral expression. A 2-10 weeks incubation period, depending on the transfected gene and the target structure. This can be combined with the head-

post implantation but not with other implantation procedures, to reduce the risk of infection in craniotomies. In the case of microscopy techniques such as mini-camera imaging, these require sufficient expression of the injected viral construct and the availability of fluorescent signals at surgery for implantable components (lenses, cranial windows) to be correctly positioned. This needs to be done in two successive steps, to ensure correct positioning of the lens and the microscope
In general, we will strive to minimize the number of surgeries necessary.
Animals that undergo viral injections need to remain in quarantine in independently ventilated cages for a 1-3 week periods, according to GGO regulations.

Manipulations
The animals will be treated with acute intraperitoneal injection of a low dose of clonazepam (0.05 mg/kg) or with antiepileptic drug, levetiracetam, acutely 200 mg/kg i.p., or chronically 75 mg/kg daily administered by miniosmotic pumps (e.g. Alzet miniosmotic pumps http://www.alzet.com/) for 28 days. The dosing is based on previous animal and human experiments for controlling hyperactivity in memory decline (29,30,42). Intracortical bipolar stimulation is achieved by stimulating the deep part of the electrode in the cortex (11). Optogenetical manipulation is achieved by injecting once viral vectors carrying a gene construct for light sensitive ion channel (12). Additionally, animals are equipped with small chronic optic cable during electrode implantation (13). Pharmacogenetical manipulation will be based on A DREADD, designer receptor, which will be expressed in neurons. Since the HM4Di has no natural ligands and respond exclusively to a synthetic small molecule, clozapine N-oxide (CNO), administration once or several times of CNO provides us a temporally and spatially specific method to control hyperactive (13).

Training/ Recordings
Behavioral experiments: All tasks used are of an appetitive nature. Training is done gradually and with positive reinforcement to support the animals' habituation to the training/recording setup and task.
Initially, animals are habituated to restraint and to head fixation, and then trained in the specific task of interest. Animals might be mildly food or water restricted to motivate behavioral performance by rewarding the animal with food or fluid reward. All food restricted animals will be monitored closely to ensure sufficient food intake. To protect neural implants or to ensure equal food availability and sufficient food intake, animals with implants will need to be housed singularly. We have tried it before with rats and mice. If rats start to argue the implant is among the first thing they bite. We saw it and there was pieces bit off from the head implant. Also with mice we have had the same issues. Since it is impossible to prevent the fighting and nibbling it is too risky to keep 2 animals in the same cage. Even one bite can destroy (reference or ground) the whole recording system and consequently I need to sacrifice the animal.

Training continues between 1 day and several weeks (max. 12 weeks with an animal) for more complex tasks but generally until a certain behavioral criterion is reached (e.g. 70% trials correct in 1 session). Sessions usually last between 30 minutes and 2-3 hours per day, where care is taken to only continue the session while the animal is accepting the head fixation.

During semi-acute and acute electrophysiological recording sessions, electrodes are inserted into the brain structures of interest and recordings are performed. Due to the lack of sub-dural pain receptors, mice usually take very little notice of this procedure even without anesthesia and analgesia.
Optical imaging can usually be done by placing the imaging microscope or mini camera over the previously prepared imaging area and don't require additional invasive procedures.
Recording session, electrical and optical, will take place daily, either after behavioral criterion is reached, or during training, depending on the task.

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

For each experimental group, the number of animals will be assessed by power analysis. Power analysis will take into account the high yield in terms of recorded cells of our methodologies. For each measure, the appropriate N number will be selected, which may be number of animals, or number of recorded neurons, or number of electrodes, etc. Because these are novel procedures, some pilot experiments will be needed to assess the effect sizes before a proper analysis can be performed. When possible we will use data from similar experiments in the literature for an initial guess of the effect sizes. Number of animals will be increased to allow for dropout due to non-functioning implants, problems at surgery, or other causes of experimental failure. The maximum group size for each group, based on our experience, will be 35 animals (15 wild types + 20 transgenic).

By using combinations state-of-the-art high-yield recording techniques, we constantly increase the number of neurons recorded per animal, and hence reduce the total amount of animals needed per group. In all cases, we expect a dropout rate for these complications of about 30% for both transgenic and wild types. In addition, we expect additional maximum 20 % dropout rate for only to transgenic animals because of the plausible epileptic activity (see the Coherence section).

B. The animals

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

The hypotheses will be tested with male mice carrying human APPswe and PS1dE9 (APdE9) mutations which develop amyloid plaques beginning at 3-4 months of age without memory dysfunction. These animals show memory impairment in memory tasks around 10-12 months of age when these animals also suffer from severe amyloid plaque burden (9–11). These mice show increase in epileptic seizures between 3 and 4.5 months of age reaching eventually a prevalence of 60% of the population (3). At this age, as we have shown, mice also display cortical and-thalamic hyperactivity (4). Therefore, studying the young animals may lead to the detection of early EEG biomarkers for early stages of AD, and studies with older animals to the characterization of the pathology of the cognitive loss.

For breeding 100 transgenic male are used.

Group size will be determined by power analysis as explained above. Each group will include transgenic animals and wild type litter mates as control animals as needed for the specific question. The maximum group size for each group, based on our experience, will be 35 animals per group (20 transgenic and 15 wild type littermates as determined by power analysis). There will be 3 groups with both early and late stage mice, so the total amount of animals will be 210 animals. The detailed description of groups is provided in the figure 1 and in the table 2 (groups 9-11).

Life stages

The experiments will be performed in mice starting from 4-6 weeks until up to 18 months.

Table 2. Description of manipulations and procedures, and the discomfort level in different experimental groups. The head fixed animals are marked as the groups 9-11.

Groups #	Experiment	Manipulations and procedures				Discomfort	Number of animals	
Number of animals total 210 **Out of which** • **Moderate discomfort: 140** • **Severe discomfort: 70**		Surgical procedures If 2 -> SEVERE If 1 -> MODERATE						
		Virus injection	Elect. implantation	Manipulation of neural activity	Behavioral test		Young	Old
9	Electrophysiological characterization of neuronal activity and pharmacological manipulation	NO	YES	YES	YES	Cumulative: MODERATE	35	35
10	Electrophysiological characterization of neuronal activity and optogenetical/pharmacogenetical manipulation	YES	YES	YES	YES	Cumulative: SEVERE	35	35
11	Electrophysiological characterization of neuronal activity and electrical / ultrasound manipulation	NO	YES	YES	YES	Cumulative: MODERATE	35	35

Species	Origin	Maximum number of animals	Life stage

Mouse wt	own breeding	45	early stage 2-10 m
mouse tg	own breeding	60	early state 2-10 m
mouse tg	own breeding	60	late stage 11-18 m
mouse wt	own breeding	45	late stage 11-18 m
mouse tg	own breeding	100	for breeding until 14 months

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that were used in one memory task may, when in good health conditions and when experimentally appropriate, be re-tested in a another memory task, helping reducing the number of animals used in total

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

[] No
[X] 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 At the moment, there are no alternative techniques capable of providing data about the relationship between behavior, learning, genetic modifications, and neural code. While our group has also expertise in computational modeling of neural systems, which are part of our research approach, for the questions that we target here there are too many missing data about biological neural network behavior for a purely theoretical approach.
We use rodents because the procedures for electrophysiological recordings, optogenetic stimulation and calcium/voltage sensitive dye imaging have been best fine-tuned in this species and to avoid use primates. Mice can perform complex memory tasks that cannot be performed by lower species while most of the brain areas know to be involved in these tasks have homologous counterparts in humans.

Reduction. It is to be noted that the high data-yield of our techniques permits a significant reduction in the number of animals needed to attain statistical significance in neurophysiological measures. Collecting the same amount of data with more traditional methodologies may require many times more animals.
The number of animals will be determined by power analyses which will take the expected experimental yield of our techniques in terms of recorded neurons into account.

Refinement The electrophysiological and behavioral techniques employed are established in the lab. Surgeries will employ advanced pain control (see below) and sterility techniques. Implantable devices are engineered (in many cases in-house) to obtain the lowest weight, smallest bulk, and maximum comfort for the animals, and are comparable with the state-of-the-art in the field in this respect. We will continue to refine our surgical techniques and devices, in order to minimize animal discomfort and adverse health consequences, as we have done in the past.

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

All animals will receive intensive pre and postsurgical care including anti-analgesic treatment, daily check of their health status and environmental enrichment. Anesthesia will be administered under monitoring of vital parameters (spO2, heart rate).
Animals will gradually be habituated to the head fixed setup e.g.according to protocols developed by our collaborator.
During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Habituation to head-fixation will be done gradually and with positive reinforcement in a low-light environment. In our experience mice usually adapt quickly to this treatment and have been shown exhibit normal foraging, food consumption, grooming and sleeping behavior.

We use transgenic animals and genetically engineered viral vectors (of low-risk class) according to GGO national regulations, for prevention of human and environmental contamination.

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.

Not applicable

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

After the surgery the animals are housed in individual cages because of veterinary concerns regarding animal well-being related to allow a short term recovery post-operatively and restrict a long term aggressive behaviour of cage mates towards animal with chronical implant. Related the behavioral task mild water or food restriction will be applied in individual housing.

G. Location where the animals procedures are performed

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

[X] No > Continue with question H.
[] Yes > Describe this establishment.

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

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

H. Pain and pain relief

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

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

Surgeries will require anesthesia and pain relief
Rodents will be anesthetized with injection drugs, e.g. ketamine/ medetomidine or urethane (for non-recovery procedures) or gas (e.g. isoflurane) during surgeries, according to state-of-the-art accepted protocols, developed in collaboration with the IvD and animal facility personnel. Atropine will be administered to support respiratory function during surgeries. Analgesia will be guaranteed with carprofen, meloxicam or other approved agents during and after surgeries.

I. Other aspects compromising the welfare of the animals

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

Breeding: Breeding might cause mild discomfort for breeding animals

Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

Handling stress: animals will be gradually habituated to handling.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (for mice: never below 90% body weight, restriction only in the hours right before the experiment).
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APdE9 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint. In most cases the risk for discomfort is moderate at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

Explain why these effects may emerge.

Breeding: Breeding might cause mild discomfort for breeding animals

Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

Handling stress: animals will be gradually habituated to handling.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (for mice: never below 90% body weight, restriction only in the hours right before the experiment).
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APdE9 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint. In most cases the risk for discomfort is moderate at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

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

Breeding: Breeding might cause mild discomfort for breeding animals

Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

Handling stress: animals will be gradually habituated to handling.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (for mice: never below 90% body weight, restriction only in the hours right before the experiment).
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.

Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APdE9 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint. In most cases the risk for discomfort is moderate at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

J. Humane endpoints

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

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

The following general humane endpoints will be applied:

1) When recovery after surgery is insufficient. In our experience, animals may lose some weight in the days immediately following the surgery. However, it will be considered if the animal shows permanent weight loss (more than 20% of the weight after the surgery for more than 7 days). In the case of juvenile animals, a lack of weight gain compatible with the animal life stage will be also considering a sign of impaired well-being.
2) When there are post-surgical complications, e.g. infection in the incision.
3) If pathogenesis without hope of recovery is determined. Visible signs of pathogenesis will be monitored such as infection, and condition of the fur. The following will be considered as signs of unhealthy state of the animal:
a. Aberrant behavior (e.g. freezing)
b. Shock
c. Dehydration
d. Weight loss
e. Nose and Mouth discharge
f. Bleeding
g. Fits/Seizures as described previously
h. Diarrhea

Should these unhealthy symptoms appear we will contact the vet to examine possible causes and monitor the animal closely to observe any improvement or decline in the condition of the animal. The vet will be consulted to judge the likelihood of recovery given the nature of the symptoms and their development. If recovery is judged unlikely or to involve prolonged suffering, then the humane endpoint will be reached.

4) When electrophysiological readings show that the head-plate is no longer firmly in place. This will be shown by a disruption of the normally stable background noise and an inability to stably record cells for a number of consecutive sessions. A recovery operation is then no longer possible.

5) When the animal is not learning the behavioral task after repeated attempts. Even if there is no worse discomfort conditions than foreseen, the experiment will be terminated, to prevent pointless manipulation

Indicate the likely incidence.

1: <10%
2: <5%
3: <5%
4: <5%
5: <5%

total < 30%

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).

Breeding: Mild, breeding animals

Handling stress: mild groups 9-11

Injection: mild, groups 10-11

Surgery and recovery: moderate groups 9 and 11, severe 10

Cumulative discomfort: moderate groups 9 and 11, severe 10

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

The animal will be killed as part of the procedure to allow us to perform perfusion and histological analysis.

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

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

Appendix
Description animal procedures

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure Circuit manipulation in freely moving mice

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The aim of these experiments is to investigate dynamics of memory within and between (interacting) relevant brain areas such as the hippocampus and neocortex in freely moving mice during (memory-) behavioral tasks. Although the use of head-fixed animals is motivated by several benefits as explained in [appendix 1], in terms of the relative ease by which complex experimental configuration may be achieved, the use of freely moving animals has the significant advantage of testing animals in a realistic or natural environment that cannot be achieved in a head-fixed setting, such as testing memory that involves direct exploration of an object or certain tasks involving spatial navigation, which heavily depends on vestibular inputs. For example it is known that neural correlates of spatial location in the hippocampus are quite different in a head-restrained, virtual reality condition compared to the freely moving situation.

In these series of experiments, we will combine state-of-the-art techniques to measures neural correlates of memory-dependent behavior to provide novel insight that will further our understanding in basic memory processes but also significantly contribute to our understanding of complex diseases and neuropsychiatric disorders where a memory deficit is a key feature.

The primary outcome measurements of these experiments are:
1. Behavioral readouts of freely moving animals in memory-related tasks for instance delayed T-test or object recognition task in which we will study various stages of memory, namely encoding, retention, consolidation and retrieval and how they are effected by progressive amyloid pathology. These tasks will involve spatial memory, recognition memory and working memory. The readout will involve e.g. percentage of trials correct, latency to response and time spent exploring.
2. Electrophysiological recordings of local and global level neural activity in memory-relevant brain for instance the hippocampus, entorhinal cortex, barrel cortex and thalamus areas to study neural correlates on a microcircuit level and the network level.
3. In vivo calcium activity measurements of memory-related areas to study individual cell activity during memory tasks with high spatial and temporal resolution
4. Optogenetic, pharmacogenetical or electrical stimulation manipulation of brain activity to study how increasing or suppressing activity in one brain area affects the behavior of the animal and how this affects activity in interacting brain areas. Stimulation may be delivered at arbitrary times, or triggered by the detection of specific patterns in neural activity
5. Measurement of the behavioral and neurophysiological effects of pharmacological manipulations, local or systemic
6. Ex-vivo Histological measures of gene expression, concentrations of relevant chemicals (e.g. neuromodulators, neurotransmitters), neuroanatomy, validation of implant placement.

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

The aim of the experiments is to investigate memory dynamics in vivo by using freely moving mice to manipulate and record or image brain activity in relevant brain areas during memory-relevant tasks. Behavioral procedures will depend on the state of the literature in this rapidly evolving field at the moment of the beginning of each experiment, and on the data we collect in previous experiment, but examples of envisaged experiments are:

- the characterization of spatial responses in the hippocampal formation and how they respond to behavioral as well as pharmacological, pharmacogenetical and optogenetic manipulations
- Recognition (e.g. of objects) memory tasks

In all cases, we will measure spontaneous ongoing behavior, or will reward the desired responses with an appetitive reward. In this procedure, no aversive conditioning (e.g. foot shocks) are foreseen.

Preparation and surgery

Surgeries: stereotaxic surgery will be required for brain implants, used to record and manipulate brain activity.
We will implant the following types of devices

1) electrode arrays/electrode probes
2) optic fibers for optogenetic stimulation
3) miniature lenses for microendoscope imaging

Depending on the specific projects and complexity of the procedures, these preparations can be either performed in one or more (max. 3) separate stereotactic surgeries. A second surgery is required as some steps require an interval of weeks between them. Injections of viral vectors (used in all groups), for example, has to be performed in advance to allow sufficient time for viral expression. A 2-10 weeks incubation period, depending on the transfected gene and the target structure. This cannot be combined with other implantation procedures, to reduce the risk of infection in craniotomies, if too long a time elapses between the implantation and the
In the case of microscopy techniques such as mini-camera imaging, these require sufficient expression of the injected viral construct and the availability of fluorescent signals at surgery for implantable components (lenses, cranial windows) to be correctly positioned. This needs to be done in two successive steps, to ensure correct positioning of the lens and the microscope. These groups will underlie three surgeries

Under certain circumstances it will be necessary to put the animal under anesthesia to repair or improve the efficiency of the implant.

To protect neural implants or to ensure equal food availability and sufficient food intake, animals with implants will need to be housed singularly.

Manipulations
Animals are subjected to pharmacological, optogenetical, pharmacogenetical or electrical manipulation to restore and/or prevent abnormal neuronal activity. In the goal two the aim is to characterize the mechanism of action of different treatments for AD related hyperactivity. The animals will be treated with acute intraperitoneal injection of a low dose of clonazepam (0.05 mg/kg), or with antiepileptic drug, levetiracetam, acutely 200 mg/kg i.p., or chronically 75 mg/kg daily administered by miniosmotic pumps (e.g. Alzet miniosmotic pumps http://www.alzet.com/) for 28 days. The dosing is based on previous animal and human experiments for controlling hyperactivity in memory decline (7,8,19). Alternatively, intracortical bipolar stimulation is achieved by stimulating the deep part of the electrode in the cortex (20). Optogenetical manipulation is achieved by injecting viral vectors carrying a gene construct for light sensitive ion channel. Additionally, animals are equipped with small chronic optic cable during electrode implantation (21). Ultrasound stimulation of brain areas will be achieved by an integrated focused ultrasound system was used (22). Pharmacogenetical manipulation will be based on A DREADD, designer receptor, which will be expressed in neurons. Since the HM4Di has no natural ligands and respond exclusively to a synthetic small molecule, clozapine N-oxide (CNO), administration of CNO provides us a temporally and spatially specific method to control hyperactive (23).

Behavioral training
Animals will be trained in several memory- or sensory-related tasks. Some of these tasks will require food or water restriction for optimal performance of the animal. Training of the animals will be conducted 5-6 days per week and depending on the task will require to be trained until the animal has reached criterion (>80% trials correct).
Optical or electrical manipulations will be performed during various phases of the memory task, such as encoding, retention, consolidation and retrieval. The readout will consist of the behavioral performance, electrophysiological recordings and/or imaging of cell activity.

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

For each experimental group, the number of animals will be assessed by power analysis. Power analysis will take into account the high yield in terms of recorded cells of our methodologies. For each measure, the appropriate N number will be selected, which may be number of animals, or number of recorded neurons, or number of electrodes, etc. Because these are novel procedures, some pilot experiments will be needed to assess the effect sizes before a proper analysis can be performed. When possible we will use data from similar experiments in the literature for an initial guess of the effect sizes. Number of animals will be increased to allow for dropout due to non-functioning implants, problems at surgery, or other causes of experimental failure. The maximum group size for each group, based on our experience, will be 35 animals (15 wild types + 20 transgenic).

By using combinations state-of-the-art high-yield recording techniques, we constantly increase the number of neurons recorded per animal, and hence reduce the total amount of animals needed per group. In all cases, we expect a dropout rate for these complications of about 30% for both transgenic and wild types. In addition, we expect additional maximum 20 % dropout rate for transgenic animals because of the plausible epileptic activity (see the Coherence section).

B. The animals

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

The hypotheses will be tested with male mice carrying human APPswe and PS1dE9 (APdE9) mutations which develop amyloid plaques beginning at 3-4 months of age without memory dysfunction. These animals show memory impairment in memory tasks around 10-12 months of age when these animals also suffer from severe amyloid plaque burden (9–11). These mice show increase in epileptic seizures between 3 and 4.5 months of age reaching eventually a prevalence of 60% of the population (3). At this age, as we have shown, mice also display cortical and-thalamic hyperactivity (4). Therefore, studying the young animals may lead to the detection of early EEG biomarkers for early stages of AD, and studies with older animals to the characterization of the pathology of the cognitive loss.

Group size will be determined by power analysis as explained above. Each group will include transgenic animals and wild type litter mates as control animals as needed for the specific question. The maximum group size for each group, based on our experience, will be 35 animals per group (20 transgenic and 15 wild type littermates as determined by power analysis). There will be 8 groups with both early and late stage mice, so the total amount of animals will be 560 animals.

For breeding 300 transgenic males are used.
The detailed description of groups is provided in the figure 1 and in the table 3 (groups 1-8).

Groups #	Experiment	Manipulations and procedures						Discomfort	Number of animals	
Number of animals total 560 **Out of which** • **Moderate discomfort: 280** • **Severe discomfort: 280**		Surgical procedures If more than 1 -> SEVERE If 1 -> MODERATE				Manipulation of neural activity	Behavioral test		Young	Old
		Virus injection	Electrode implantation	Lens impalntation Lens surgery	Lens base surgery					
1-4	Electrophysiological characterization of neuronal activity and pharmacological / electrical manipulation	NO	YES	NO	NO	YES	YES	Cumulative: MODERATE	4x35 =140	4x35 =140
5	Electrophysiological characterization of neuronal activity and optogenetical or pharmacogenetical manipulation	YES	YES	NO	NO	YES	YES	Cumulative: SEVERE	35	35
6-8	Calcium imaging of neuronal activity and pahramacological or optogenetical or pharmacogenetical manipulation (Implantation of calcium imaging lens needs 2 operations!, 3 surgical procedures in these groups)	YES	NO	YES	YES	YES	YES	Cumulative: SEVERE	3x35 =105	3x35 =105

Life stages
The experiments will be performed in mice starting from 4-6 weeks until up to 18 months.

Species	Origin	Maximum number of animals	Life stage
mouse wt	own breeding	120	early stage 2-10 months
mouse tg	own breeding	160	late stage 11-18
mouse tg	own breeding	160	early stage 2-10 months
mouse wt	own breeding	120	late stage 11-18
mouse tg	own breeding	300	for breeding till 14 months

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that were used in one memory task may, when in good health conditions and when experimentally appropriate, be re-used for a different memory task, helping reducing the number of animals used in total. In no case will an animal be measured for more than 2 months, or be in an experiment after 1 year of age. Animals experiencing discomfort beyond moderate will not be re-used.

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

[] No
[X] 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 At the moment, there are no alternative techniques capable of providing data about the relationship between behavior, learning, genetic modifications, and neural code. While our group has also expertise in computational modeling of neural systems, which are part of our research approach, for the questions that we target here there are too many missing data about biological neural network behavior for a purely theoretical approach.
We use rodents because the procedures for electrophysiological recordings, optogenetic stimulation and calcium/voltage sensitive dye imaging have

been best fine-tuned in this species and to avoid use primates. Mice can perform complex memory tasks that cannot be performed by lower species while most of the brain areas know to be involved in these tasks have homologous counterparts in humans.

Reduction. It is to be noted that the high data-yield of our techniques permits a significant reduction in the number of animals needed to attain statistical significance in neurophysiological measures. Collecting the same amount of data with more traditional methodologies may require many times more animals.
The number of animals will be determined by power analyses which will take the expected experimental yield of our techniques in terms of recorded neurons into account.

Refinement The electrophysiological and behavioral techniques employed are established in the lab. Surgeries will employ advanced pain control (see below) and sterility techniques. Implantable devices are engineered (in many cases in-house) to obtain the lowest weight, smallest bulk, and maximum comfort for the animals, and are comparable with the state-of-the-art in the field in this respect. We will continue to refine our surgical techniques and devices, in order to minimize animal discomfort and adverse health consequences, as we have done in the past.

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

All animals will receive intensive pre and postsurgical care including anti-analgesic treatment, daily check of their health status and environmental enrichment.
Anesthesia will be administered under monitoring of vital parameters (spO2, heart rate).
During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. However, daily periods of social interactions with former littermates can usually be arranged after training.
During this period, animals will be checked daily for their health status and will receive additional food if required.
Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.

We use transgenic animals and genetically engineered viral vectors (of low-risk class) according to GGO national regulations, for prevention of human and environmental contamination.

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.

Not applicable

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

After the surgery the animals are housed in individual cages because of veterinary concerns regarding animal well-being related to allow a short term recovery post-operatively and restrict a long term aggressive behaviour of cage mates towards animal with chronical implant. Related the behavioral task mild water or food restriction will be applied in individual housing.

G. Location where the animals procedures are performed

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

[X] No > Continue with question H.
[] Yes > Describe this establishment.

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

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

Surgeries will require anesthesia and pain relief.
Mice will be anesthetized with injection drugs, e.g. ketamine/ medetomidine or urethane (for non-recovery procedures) or gas (e.g. isoflurane) during surgeries, according to state-of-the-art accepted protocols, developed in collaboration with the IvD and animal facility personnel. Atropine will be administered to support respiratory function during surgeries. Analgesia will be guaranteed with carprofen, meloxicam or other approved agents during and after surgeries.

I. Other aspects compromising the welfare of the animals

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

Breeding: Breeding might cause mild discomfort for breeding animals
Handling stress: animals will be gradually habituated to handling.
Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (never below 90% body weight), restriction only in the hours right before the experiment.
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APP/PS1 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint.

In most cases the risk for discomfort is moderate (3) at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

Explain why these effects may emerge.

Breeding: Breeding might cause mild discomfort for breeding animals

Handling stress: animals will be gradually habituated to handling.
Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment. Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (never below 90% body weight), restriction only in the hours right before the experiment.
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APP/PS1 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint.
In most cases the risk for discomfort is moderate (3) at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

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

Breeding: Breeding might cause mild discomfort for breeding animals

Handling stress: animals will be gradually habituated to handling.
Individual housing: During training and recording stages, animals will receive a majority of their daily food requirements during the behavioral session. This might make it necessary to house them individually during this period to ensure equal food availability for all mice in the experiment.

Also, animals are housed individually after implantation operation to avoid cage mates to harm the implant and cause discomfort for the implanted animal.
Recovery from surgery: analgesia, control of temperature, soft food available, continuous monitoring.
Mild food or water restriction: control of weight (never below 90% body weight), restriction only in the hours right before the experiment.
All procedures with the animals will be performed by experienced researchers/caretakers to keep minimize the occurrence of post-surgical wounds.
Health status of the animals will be checked daily while in training and additional food will be provided if required.

In 65 % of APP/PS1 mice epileptic seizures are observed before the age of 5 months, which are fatal in 10-20% of the cases. In the most severe cases we will have, in consultation with the IvD, assess to exclude the animals from the experiments and consider that a humane endpoint.
In most cases the risk for discomfort is moderate (3) at most, because seizures last for a very short time (about 2-5 seconds, maximum 30 s) and the recovery is normally good, based on behavioural observations. After 6 months of age, the risk for seizures is much more limited.

However, if animal experience a seizure during the recording the animal is release immediately for the recording device to allow full undisturbed recovery.

J. Humane endpoints

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

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

1) When recovery after surgery is insufficient. In our experience, animals may lose some weight in the days immediately following the surgery. However, it will be considered if the animal shows permanent weight loss (more than 20% of the weight after the surgery for more than 7 days).
2) When there are post-surgical complications, e.g. infection in the incision.

3) If pathogenesis without hope of recovery is determined. Visible signs of pathogenesis will be monitored such as infection, and condition of the fur. The following will be considered as signs of unhealthy state of the animal:
Aberrant behavior (e.g. freezing)

Shock

Dehydration

Weight loss

Nose and Mouth discharge

Bleeding

Fits/ Seizures as described previously

Diarrhea

Should these unhealthy symptoms appear we will contact the vet to examine possible causes and monitor the animal closely to observe any improvement or decline in the condition of the animal. The vet will be consulted to judge the likelihood of recovery given the nature of the symptoms and their development. If recovery is judged unlikely or to involve prolonged suffering, then the humane endpoint will be reached.

4) When electrophysiological readings show that the head-plate is no longer firmly in place. This will be shown by a disruption of the normally stable background noise and an inability to stably record cells for a number of consecutive sessions. A recovery operation is then no longer possible.

5) when the animal is not learning the behavioral task after repeated attempts. Even if there is no worse discomfort conditions than foreseen, the experiment will be terminated, to prevent pointless manipulation

Indicate the likely incidence.

1: <10%
2: <5%
3: <5%

4: <5%
5: <5%

total < 30%

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).

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).
Breeding: Mild, breeding animals
Handling stress: mild, groups 1-8

Injection: mild, groups 1-8

Surgery and recovery: moderate groups 1-4, severe groups 5-8

Cumulative discomfort: moderate groups 1-4, severe groups 5-8

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

The animal will be killed as part of the procedure to allow us to perform perfusion and histological analysis.

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

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

7.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen
[redacted]
T.a.v. [redacted]
Postbus 9101
6500 HB NIJMEGEN

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

Onze referentie
Aanvraagnummer
AVD1030020171327

Bijlagen
2

Datum 7 april 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 7 april 2017. Het gaat om uw project "Alzheimer's disease and hyperactivity". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD1030020171327. Gebruik dit nummer wanneer u contact met de CCD opneemt.

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

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

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

Datum:
7 april 2017
Aanvraagnummer:
AVD1030020171327

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
7 april 2017
Aanvraagnummer:
AVD1030020171327

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	10300
Naam instelling of organisatie:	Stichting Katholieke Universiteit Nijmegen
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	41055629
Straat en huisnummer:	Geert Grooteplein 29
Postbus:	9101
Postcode en plaats:	6500 HB NIJMEGEN
IBAN:	NL90ABNA0231209983
Tenaamstelling van het rekeningnummer:	UMC St Radboud

Gegevens verantwoordelijke onderzoeker

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

Datum:
7 april 2017
Aanvraagnummer:
AVD1030020171327

Gegevens plaatsvervangende verantwoordelijke onderzoeker

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

Gegevens verantwoordelijke uitvoering proces

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

Over uw aanvraag

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

Over uw project

Geplande startdatum:	8 mei 2017
Geplande einddatum:	7 mei 2022
Titel project:	Alzheimer's disease and hyperactivity
Titel niet-technische samenvatting:	Hyperactiviteit en de ziekte van Alzheimer
Naam DEC:	RU DEC
Postadres DEC:	Postbus 9101, 6500 HB Nijmegen [redacted]
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.287,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

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

Ondertekening

Naam:	[redacted]
Functie:	Instantie voor dierenwelzijn
Plaats:	Nijmegen
Datum:	7 april 2017

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Geert Grooteplein 29
Postbus 9101, [redacted]
6500 HB NIJMEGEN
[redacted]

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

Onze referentie
Aanvraagnummer
AVD1030020171327

Bijlagen
2

Datum 7 april 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 7 april 2017
Vervaldatum: 7 mei 2017
Factuurnummer: 171327
Ordernummer: Kostenplaats en kostensoort: [redacted]
projectnummer: 2016-0098 Verantwoordelijk onderzoeker: [redacted]

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD1030020171327	€ 1.287,00

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

DEC-advies

A. Algemene gegevens over de procedure

1. Aanvraagnummer: 2016-0098
2. Titel van het project: Alzheimer's disease and hyperactivity
3. Titel van de NTS: Hyperactiviteit en de ziekte van Alzheimer

4. Type aanvraag:
   ☒ nieuwe aanvraag projectvergunning
   ☐ wijziging van vergunning met nummer

5. Contactgegevens DEC:
   - naam DEC: RUDEC
   - telefoonnummer contactpersoon: [redacted] bereikbaar op maandag, dinsdag, en donderdag van 9:00 tot 15:00 uur
   - e-mailadres contactpersoon: [redacted]

6. Adviestraject (data dd-mm-jjjj):
   ☒ ontvangen door DEC: 20-01-2017
   ☐ aanvraag compleet
   ☒ in vergadering besproken: 07-02-2017 en 07-03-2017
   ☐ anderszins behandeld
   ☒ termijnonderbreking(en) van 13-02-2017 tot 18-02-2017, en van 13-03-2017 tot 15-03-2017
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☒ aanpassing aanvraag: 18-02-2017 en 15-03-2017
   ☒ advies aan CCD: 06-04-2017

7. De inhoud van dit project is afgestemd met de IvD en deze heeft geen bezwaren tegen de uitvoering van het project binnen deze instelling.

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

9. Correspondentie met de aanvrager
   - Datum vragen: 13-02-2017
   - Datum antwoorden: 18-02-2017
   - Gestelde vragen en antwoorden:

     General information

     -1.3 AD graag voluit schrijven.

     Antwoord: Corrected

     Project Proposal:

     -3.1 Het verband tussen amyloïde plaques, hyperactiviteit en geheugenstoornissen is nog niet vastgesteld. Er zijn meerdere hypothesen die dit verband beschrijven. Het zou bijvoorbeeld zo kunnen zijn dat de amyloïde plaques leiden tot hyperactiviteit van hersencellen die vervolgens leidt tot aantasting van het geheugen. Het zou ook kunnen dat de hyperactiviteit aan de basis staat van zowel aantasting van het geheugen als plaquevorming. Een aantal onderzoekers is er van overtuigd dat de plaques niet belangrijk zijn voor het ontstaan van de ziekte van Alzheimer, omdat een interventie gebaseerd op het tegengaan van plaquevorming geen effect had op de ziekte. Zal de aanvrager onderzoeken of de plaques verantwoordelijk zijn voor de geheugenstoornis? De onderzoekers melden dat zij

hun hypothesen zullen onderzoeken in een muismodel, maar welke hypothesen zij bedoelen is nog onvoldoende benoemd.
Antwoord: We have provided some additional information concerning the role of amyloid pathology and hyperactivity, see sections 3.1. and 3.2. . This project is focused on abnormal hyperactivity in single cell and neuronal network level. It seems that this activity is most severe during pre-plaque phase even before cognitive decline. However, the role of abnormal activity during plaque phase is still under investigation and this project aims to also characterize abnormal activity during plaque phase. However, manipulation of amount of amyloid load and whether this has an effect on cognitive decline or memory problems is not in the scope of this project.
-3.1 De onderzoekers noemen enkele publicaties waaruit een verband tussen abnormale excitatoire neuronale activiteit (hyperactiviteit) en de ziekte van Alzheimer zou kunnen blijken. Zijn er geen gegevens over het optreden van epilepsie bij patiënten met de ziekte van Alzheimer? Is de hyperactiviteit ooit gemeten bij patiënten met deze ziekte? Verwachten de onderzoekers dat dit bij alle patiënten met Alzheimer optreedt, of bij een specifiek deel van de patiëntenpopulatie?
Antwoord: We have provided additional information concerning the translational value of this project in the section 3.1. Shortly, the correlation of AD related amyloid pathology and epilepsy is based on epidemiological data showing that patients with aMCI who had epilepsy presented with symptoms of cognitive decline even 7 years earlier than patients with aMCI who did not have epilepsy. Additionally, patients with AD who had epilepsy presented with cognitive decline 6 years earlier than patients with AD who did not have epilepsy. In addition, it has been shown that EEG of AD patients shows epileptiform activity and also fMRI studies support this notion showing increased activity in the hippocampal region.
-3.1 Een kale opsomming van technieken hoort niet thuis in dit onderdeel. In het onderdeel strategie kunnen de onderzoekers uiteenzetten hoe zij de doelstelling van de projectaanvraag operationaliseren.
Antwoord: This part has now been moved to the section 3.4.1.
-3.2 De onderzoekers noemen geen concreet doel van de projectaanvraag, waardoor de commissie niet kan toetsen of dit haalbaar is binnen de looptijd van het project. Welk 'comprehensive picture' bedoelen de onderzoekers? De DEC kan niet goed beoordelen of dit doel behaald kan worden door het uitvoeren van de beschreven experimenten. Het blijft bijvoorbeeld onduidelijk in welke hersengebieden de onderzoekers geïnteresseerd zijn en welke hypothesen zij willen onderzoeken (zie ook de vraag over 3.1). Relevante publicaties van de projectleider ontbreken bij de onderbouwing van de haalbaarheid.
Antwoord: The section 3.2. addressing this issue has now been rewritten and also section 3.1. has been rewritten related to the aims of this project. Additional references have been added to the application.
-3.3 De onderzoekers worden verzocht het maatschappelijk en wetenschappelijk belang beter toe te lichten. Welke in de praktijk bruikbare biomarkers verwachten zij te kunnen identificeren in dit onderzoek aan dit muismodel?
Antwoord: Section 3.3. has now been provided with additional information. As early biomarkers we mention the possibility to find EEG and/or MEG biomarkers for the early detection of AD.
-3.4.1: De onderzoekers zullen zowel jonge als oude dieren onderzoeken. Het is niet duidelijk waarom dit nodig is voor het bereiken van de doelstelling van het project: dit wordt niet genoemd bij 3.2.
Antwoord: The issue of using different age animals in this project has now been addressed in the section 3.4.1. Shortly, younger preplaque animals show the most severe abnormal neuronal activity, on the hand, older animals with amyloid plaques show cognitive and memory dysfunction. Therefore, characterization of neuronal activity and the mechanism of

action of different manipulations at different ages provide complementary picture hyperactivity during amyloid accumulation.
-3.4.2: Welke hersengebieden worden getarget? Is de keuze gebaseerd op uitkomsten van DAP1?
Antwoord: We have provided more detailed information of the brain areas mainly focused. The areas are originally selected based on our previous results and literature being the most important brain areas of cognitive functions and also affected by the amyloid related hyperactivity. During the project with more detailed characterization of single cell and neuronal network activity we will select from original brain areas only the areas which seems to be mostly affected by amyloid accumulation. See the section 3.4.1.
-3.4.2: Het project kent meerdere verschillende doelen die echter nergens worden benoemd. Welke doelen bedoelt de aanvrager (zie ook de vraag over 3.2)? Deze doelstellingen zijn in principe de aanknopingspunten voor de experimenten die uitgevoerd zullen worden. In de figuur staan nu alleen technieken, maar niet de verschillende onderzoeksstappen die nodig zijn voor het behalen van het einddoel. In figuur twee (onderdeel 3.4.3) wordt een overzicht gepresenteerd van de technieken die op verschillende onderzoeksgroepen worden toegepast. Het blijft onduidelijk waarom het toepassen van deze technieken zal leiden tot het bereiken van de doelstelling van het project.
Antwoord: The terminology has been corrected throughout the text and clear objectives are now provided in the section 3.2. A new table explain the work packages derived from the objectives has been added with go-no-go-criteria, see table 1.
-3.4.3: Bij onderdeel 3.4.2 is sprake van 'goal one' en 'goal two'. Worden de resultaten uit 'goal one' gebruikt voor het design van de experimenten voor 'goal two'? De criteria op grond waarvan het project wel of niet zal worden gecontinueerd zijn niet duidelijk vermeld.
Antwoord: See the previous comment.

Description of Animal Procedures:
***DAP1**
-A: De geheugentaken die de onderzoekers zullen gebruiken zijn nog niet gespecificeerd.
Antwoord: The memory tasks used in this project has now been described, see section A.
-A: Welke hersengebieden willen de onderzoekers bestuderen?
Antwoord: This part has been added, see section A and 3.4.2
-A: De beschrijving van de experimenten in deze bijlage is erg algemeen gehouden. De commissie kan op basis hiervan niet goed inschatten wat elk dier zal ondergaan. Wellicht kan een figuur of tabel duidelijk maken wat er in de onderscheiden onderzoeksgroepen met welke technieken onderzocht zal worden, welke handelingen (bijv. hoeveel operaties!) de dieren zullen ondergaan en welk cumulatief ongerief dit voor de dieren in de verschillende groepen zal opleveren.
Antwoord: A more detailed description of this part has been added already in the general part, see sections 3.1. and 3.2. In addition, a new table with detailed information has been added, see table 2.
-A2: Het geven van voedsel of water dat eerder is achtergehouden om het dier te motiveren, is niet echt op te vatten als een beloning.
Antwoord: The term reward is removed from the text.
-A2: Hersteloperaties zijn niet gebruikelijk bij muizen.
Antwoord: Clarified the text. If animals implant is severely damaged, no reversal operation will be conducted.
-B: Worden dieren van beide geslachten in gelijke aantallen gebruikt?
Antwoord: Corrected. Only males.
-B: In de tabel is sprake van 2 operaties, terwijl in de tekst wordt vermeld dat er maximaal 3 operaties zullen plaatsvinden. S.v.p. in overeenstemming brengen met elkaar.

Antwoord: This has been corrected. See tables 2 and 3.
-B: De fokdieren met ongerief die nodig zijn om experimentele dieren te verkrijgen dienen in elke bijlage opgenomen te worden.
Antwoord: This has been corrected. Appendix 1 (head fixed) 150 male tg mice, and appendix 2 (freely moving) 350 tg males.
-C: De vraag betreft hergebruik van dieren uit een andere vergunning. Alle dieren zullen matig ongerief ondervinden van de operatie, dus het is onduidelijk wat de onderzoekers met deze opmerking bedoelen.
Antwoord: This comment has been removed.
-I: De onderzoekers zullen behalve voedselrestrictie misschien waterrestrictie gebruiken. Dit laatste is hier niet vermeld.
Antwoord: Water restriction added.
-I: De individuele huisvesting (zoals gemeld bij D2) ontbreekt bij de opsomming. En de reden voor individuele huisvesting in DAP2 (onderdeel F)is niet helder omgeschreven.
Antwoord: Individual housing added with more clarification.
-K: Het ongerief door het fenotype van de dieren is niet adequaat beschreven. Bovendien veroorzaken handelingen met de APdE9 muizen meer ongerief omdat deze dieren stressgevoeliger zijn. Hoe verwacht de onderzoeker dat dieren met dit fenotype zich gedragen tijdens zo'n experiment? Wat gebeurt er wanneer een muis een seizure krijgt in de meetopstelling?
Antwoord: The breeding of these animals is done in the house by a group of multiple years of experience. We are using the same breeding protocols also in this project. These animals show slight hyperactivity and might be more vulnerable to stress. Therefore, these animals are handled throughout the whole project by experienced researchers with even 10 years of experience of this mouse model. We take this detail into account in all steps of the experiments and adjust the procedures accordingly if needed. Description of the procedure in case of seizure during recording has been added, see section I.
-K: De commissie is van mening dat het ongerief voor de dieren die 2-3 maal een stereotactische operatie ondergaan in combinatie met de overige handelingen ernstig is.
Antwoord: The categorization of the severity has been corrected.

***DAP2**
De onderzoekers worden verzocht na te gaan welke opmerkingen voor DAP1 ook van toepassing zijn voor DAP2.
Antwoord: All the previous questions and comments have also been addressed accordingly in the appendix 2.

Niet-technische samenvatting:
-1.1:De titel past onvoldoende bij de projectaanvraag.
-3.2: S.v.p. aanpassen in overeenstemming met de herziene tekst n.a.v. de vraag van de commissie
-3.4: Transgene dieren zouden ongerief kunnen hebben (i.p.v. mogen).
-3.5 De commissie verzoekt u de tweede zin te verwijderen.
-4.3: De commissie meent dat de onderzoekers niet van plan waren om dit bij apen te onderzoeken. Zij raadt hen aan de laatste zin te schrappen.
-De onderzoekers worden verzocht te checken of de beantwoording van bovenstaande vragen over het Project Proposal en de DAPs ook leidt tot aanpassingen in de NTS
Antwoord: This section has been corrected and the changes in the othes parts have also been addressed by help of a native Dutch speaking person.

- Datum vragen: 13-03-2017

- Datum antwoorden: 15-03-2017

Description of Animal Procedures:
-B: Waarom willen de onderzoekers alleen mannetjes gebruiken? De onderbouwing hiervoor ontbreekt nog.
See correction in the 3.4.2. "The experiments are conducted in male mice. The scope of this research is not in the role of hormonal effect on the AD pathology. Because there is gender differences in amyloid burden accumulation and overall AD pathology is strongly linked to the fluctuation in the estrus cycle, we will not use female mice (24, 25)."
See also additional references 24 and 25.
-B: De vraag over het aantal operaties is nog niet adequaat beantwoord. In de tekst (onderdeel A2) worden nog steeds 3 operaties genoemd, terwijl in tabel 2 sprake is van 1 of 2 operaties.
The figure has been corrected accordingly and number of operation is now emphasized.

Niet-technische samenvatting:
- De vragen van de commissie over de NTS zijn niet beantwoord, en de aanpassingen in het project zijn nog niet doorgevoerd in de NTS.
There seems to be misunderstanding. These issues have been considered in the public summary even though in the process the labelling of corrections is missing. However, now the corrections have been marked as red.
Just to make sure and clear, I also comment the changes done in the previous version in this cover letter.
-Onderdeel 3.1 begint met een vraag die de commissie niet kan plaatsen.
The questions has been removed.
-1.1: De titel past onvoldoende bij de projectaanvraag.
The headline has been corrected "Hyperactiviteit en de ziekte van Alzheimer"
-3.2: S.v.p. aanpassen in overeenstemming met de herziene tekst n.a.v. de vraag van de commissie
This section has been corrected accordingly ("van biomarkers (electroencephalography en magnetoencephalography).").
-3.4: Transgene dieren zouden ongerief kunnen hebben (i.p.v. mogen).
Indication of plausible epileptic seizures has been added.
-3.5 De commissie verzoekt u de tweede zin te verwijderen.
This has been deleted
-4.3: De commissie meent dat de onderzoekers niet van plan waren om dit bij apen te onderzoeken. Zij raadt hen aan de laatste zin te schrappen.
This mention has been removed
- De onderzoekers worden verzocht te checken of de beantwoording van bovenstaande vragen over het Project Proposal en de DAPs ook leidt tot aanpassingen in de NTS.
The overall coherence of the text has been checked.

- De antwoorden hebben geleid tot aanpassing van de aanvraag

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

B. Beoordeling (adviesvraag en behandeling)

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

2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er is geen betrokkenheid van DEC-leden bij deze projectaanvraag, waardoor onafhankelijkheid en onpartijdigheid zijn gewaarborgd.

C. Beoordeling (inhoud)

1. Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. De opzet komt het best overeen met voorbeeld 1 uit de handreiking 'Wat is een project'. De verschillende subdoelen zijn noodzakelijk om de doelstelling te behalen. Het is niet mogelijk om de individuele doelen te toetsen, omdat er sprake is van onderlinge afhankelijkheid. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. De aanvrager heeft, zowel binnen de doelstellingen en bijlagen dierproeven, als tussen de doelstellingen, beschreven op basis van welke criteria zij zal besluiten het project wel of niet te continueren. De DEC is er daardoor van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. Gezien bovenstaande is de DEC van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.

2. Voor zover de DEC weet is er geen "tegenstrijdige" wetgeving die het uitvoeren van de experimenten in de weg zou kunnen staan.

3. De in de aanvraag aangekruiste doelcategorieën zijn niet geheel in overeenstemming met de hoofddoelstelling. De onderzoekers geven aan dat het translationeel onderzoek is, maar de commissie vindt dit discutabel. Het veronderstelde verband tussen hyperactiviteit en de ziekte van Alzheimer is immers nog niet onomstotelijk vastgesteld.

   Belangen en waarden

4. Het directe doel van het project is de link tussen elektrische activiteit en geheugen in kaart brengen. De doelen van het onderzoek zijn: bepalen hoe hyperactiviteit en epilepsie-achtige activiteit de twee geheugen-gerelateerde netwerken (corticothalamisch en intra-hippocampal) aantasten; het tegengaan van de hyperactiviteit in deze netwerken op verschillende manieren (farmacologisch, optogenetisch, farmacogenetisch of elektrisch); het effect van het onderdrukken van pathologische hyperactiviteit op cognitieve functies onderzoeken. Het uiteindelijke doel is de identificatie van nieuwe biomarkers (EEG en/of MEG markers) die gebruikt kunnen worden voor vroege diagnose van de ziekte van Alzheimer, en voor het ontwikkelen van nieuwe behandelingen. Het veronderstelde verband tussen hyperactiviteit en de ziekte van Alzheimer is nog niet onomstotelijk vastgesteld. De DEC is daarom van mening dat er binnen dit project geen directe relatie is tussen het doel van deze projectaanvraag en het uiteindelijke doel. De aanvrager heeft duidelijk gemaakt wat de status van het onderzoeksveld is en wat de bijdrage van dit project daaraan zal zijn. Uit de aanvraag blijkt dat de kennis van de relatie tussen amyloïde plaques, epilepsie-achtige hersenactiviteit en geheugenstoornissen op dit moment zeer beperkt is, en dat meer kennis op termijn mogelijk kan bijdragen aan vroege diagnostiek en behandelmogelijkheden voor de ziekte van Alzheimer. Naar de menig van de DEC is het doel van deze projectaanvraag daarom gerechtvaardigd binnen de context van het onderzoeksveld.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.

Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.
Voor de onderzoekers geldt dat het publiceren van belangrijke nieuwe wetenschappelijke inzichten resulteert in een goede wetenschappelijke reputatie, hetgeen vaak de sleutel is voor het verkrijgen van nieuwe onderzoeksmiddelen en mogelijkheden. Dit kan door de onderzoeker zelf van belang geacht worden, maar dient naar de mening van de DEC geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren. Het gaat uiteindelijk om de vraag of dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis).
Voor patiënten en de samenleving is dit onderzoek indirect van belang, omdat het kan bijdragen aan vroegere diagnostiek en nieuwe behandelmogelijkheden voor de ziekte van Alzheimer. Vroege diagnostiek en behandeling op basis van mechanistisch inzicht kan wellicht bijdragen aan behoud van de geheugenfunctie en aan langer behoud van de zelfstandigheid van oudere mensen. Dit kan er toe leiden dat de patiënt een betere kwaliteit van leven heeft. Het kunnen beschikken over adequate behandelingen voor ernstige verouderingsziekten, zoals de ziekte van Alzheimer, is van groot belang voor de vergrijzende samenleving.

6. Er is geen aanleiding voor de DEC om de in de aanvraag beschreven effecten op het milieu in twijfel te trekken.

Proefopzet en haalbaarheid

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, zoals blijkt uit de in de aanvraag vermelde publicaties van deze onderzoeksgroep. De aanvragers beschikken over voldoende kennis en kunde om te kunnen voldoen aan alle zorgvuldigheidseisen omtrent het verrichten van dierproeven.

8. De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet en uitkomstparameters sluiten hier logisch bij aan. Bovendien heeft deze groep veel ervaring in dit onderzoeksveld en met de voorgestelde dierproeven. De DEC is dan ook van mening dat het project goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project.

Welzijn dieren

9. Er is geen sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
   ☐ Bedreigde diersoort(en) (10e lid 4)
   ☐ Niet-menselijke primaten (10e)
   ☐ Dieren in/uit het wild (10f)
   ☐ Niet gefokt voor dierproeven (11, bijlage I richtlijn)
   ☐ Zwerfdieren (10h)
   ☐ Hergebruik (1e lid 2)
   ☐ Locatie: buiten instelling vergunninghouder (10g)
   ☐ Geen toepassing verdoving/pijnbestrijding (13)
   ☐ Dodingsmethode niet volgens bijlage IV richtlijn (13c lid 3)

10. De huisvesting en verzorging van de dieren zijn conform de eisen in bijlage III van richtlijn 2010/63/EU.

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Het ongerief voor de dieren wordt vooral veroorzaakt door meerdere stereotactische operaties in combinatie met de overige handelingen. Het ongerief is juist ingeschat als licht voor 39% van de dieren (fokdieren die ongerief vanwege hun fenotype ondervinden), matig voor 33% van de dieren en ernstig voor 28% van de dieren.

12. De integriteit van dieren wordt aangetast doordat er een 'headpost' wordt aangebracht op de kop van het dier waarmee de kop kan worden gefixeerd en elektroden/optische vezels kunnen worden aangesloten op apparatuur. In de hersenen van de transgene dieren ontstaan plaques en zij hebben vaak epileptische aanvallen. Het dier wordt hierdoor gehinderd in zijn normale gedrag en de zelfredzaamheid neemt af.

13. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op het experiment. Het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken is op basis van eerdere ervaring met dit diermodel en met deze experimenten ingeschat. De commissie is het eens met deze inschatting en de gehanteerde humane eindpunten.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. Het is op dit moment niet mogelijk om de relatie tussen gedrag, leren, neurale code, en invloed van genen hierop te bestuderen zonder proefdieren. De onderzoekers maken voor het beantwoorden van andere onderzoeksvragen regelmatig gebruik van computermodellen, maar dat is voor deze onderzoeksvragen niet mogelijk.

15. Het maximale aantal te gebruiken dieren is realistisch ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet en de looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog een wetenschappelijk betrouwbaar resultaat kan worden verkregen. Door de stapsgewijze aanpak waarin de resultaten uit eerdere proeven worden gebruikt voor het design van vervolgexperimenten wordt onnodig gebruik van proefdieren voorkomen. Door de gebruikte technieken worden veel data verkregen van elk proefdier. Er zijn daardoor minder dieren nodig voor het verkrijgen van betrouwbare neurofysiologische metingen.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. Knaagdieren zijn de minst complexe diersoorten die ingewikkelde geheugentaken kunnen uitvoeren. De hersengebieden die hierbij betrokken zijn hebben homologen bij de mens, waardoor de resultaten makkelijker vertaalbaar zijn. De gebruikte testen zijn de minst belastende voor het beantwoorden van de onderzoeksvragen. De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo humaan mogelijk worden uitgevoerd.

17. Het betreft geen wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. De aanvrager zal in het project alleen gebruik maken van mannelijke dieren. De aanvrager geeft hiervoor de volgende onderbouwing: “Hormonale effecten op AD pathologie zijn geen onderwerp van studie. Aangezien er geslachtsverschillen zijn in accumulatie van amyloïd en de algemene AD pathologie sterk gelinkt is aan de fluctuaties in de oestrus cyclus zullen we geen vrouwelijke muizen gebruiken.” De DEC is van mening dat de aanvrager in voldoende mate wetenschappelijk heeft onderbouwd dat het om de doelstellingen met zo min mogelijk dieren te bereiken noodzakelijk is om de proeven met alleen mannelijke dieren uit te voeren.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om het hersenweefsel te kunnen onderzoeken voor het beantwoorden van bepaalde onderzoeksvragen. De gebruikte dodingsmethode staat vermeld in bijlage IV van richtlijn 2010/63/EU.

20. Er worden in deze projectaanvraag geen landbouwhuisdieren, honden, katten of niet-humane primaten gebruikt (en dus ook niet gedood om niet-wetenschappelijke redenen).

    NTS

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

D. Ethische afweging

1. Rechtvaardigt het belang van de doelstelling van het project het ongerief dat de dieren wordt aangedaan, en is aan alle zorgvuldigheidseisen (3V’s) voldaan?

2. Er vindt een lichte, matige of ernstige aantasting van welzijn en integriteit van de proefdieren plaats (beschreven in C9 tot C20). De doelstellingen kunnen niet zonder dieren behaald worden. De onderzoekers doen al het mogelijke om het lijden van de dieren en het aantal dieren te beperken.
   Voor de samenleving/patiënten is dit onderzoek van belang, omdat het meer kennis zal opleveren over de relatie tussen epilepsie-achtige hersenactiviteit en geheugenstoornissen. De DEC kent daar veel gewicht aan toe om de volgende redenen. Het veronderstelde verband tussen hyperactiviteit en de ziekte van Alzheimer is nog niet onomstotelijk vastgesteld. Wanneer dat verband inderdaad kan worden aangetoond dan opent dat op termijn nieuwe mogelijkheden voor de ontwikkeling van vroege diagnostiek en behandeling van de ziekte van Alzheimer. Deze ziekte komt veel voor en heeft een grote impact op de patiënten en hun directe omgeving. Een therapie om het ziekteproces substantieel te vertragen, te stoppen of om te keren is nog niet voor handen. De kennis die wordt vergaard met dit project kan bijdragen aan de ontwikkeling van een nieuwe therapie voor deze mensen. De commissie acht meer kennis over het verband tussen hyperactiviteit en de ziekte van Alzheimer van substantieel belang.

3. De DEC is overtuigd van het belang van de doelstellingen: de link tussen elektrische activiteit en geheugen in kaart brengen. Het uiteindelijke doel daarvan is de identificatie van nieuwe biomarkers (EEG en/of MEG markers) die gebruikt kunnen worden voor vroege diagnose van de ziekte van Alzheimer, en het ontwikkelen van nieuwe behandelingen. De DEC is van mening dat de belangen van de patiënten voldoende zwaar wegen om het schaden van de belangen van de proefdieren (om gevrijwaard te blijven van een aantasting van hun welzijn en integriteit) te rechtvaardigen. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De DEC is van mening dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven

zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat zij zal kunnen voorkomen dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.
De DEC is van oordeel dat het hier boven geschetste belang de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

1. Advies aan de CCD
   ☐ De DEC adviseert de vergunning te verlenen

   ☒ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
      X Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
      - Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
      - Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

   ☐ De DEC adviseert de vergunning niet te verlenen vanwege:
      - De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:…
      - De volgende doorslaggevende ethische bezwaren:…
      - De volgende tekortkomingen in de aanvraag:…

2. Het uitgebrachte advies is gebaseerd op consensus.

3. Er zijn geen knelpunten of dilemma's geconstateerd – zowel binnen als buiten de context van het project - die de verantwoordelijkheid en competentie van de DEC overstijgen.

AVD103002017327-Antwoord-(2016-0098-20-04-2017)

I would like to thank you for this valuable comments. Please find the attached comments and corrections

Additional comment

First, I would like to you to correct an unfortunate error in the section "3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed."

The total amount of animals is stated as 1310 twice even though the correct number is 1170.

Comments to the first point concerning the re-use.

Here by re-use we intended the use of previously implanted animals in a different behavioral test. No new surgical intervention will be operated on these animals.

In this sense, no new experiment will ever be performed on animal experiencing severe discomfort. All behavioral manipulation will be clearly specified in the work protocol and will comply with the stipulations in the CCD project 2016-0098, in terms of duration, number of experimental days, etc.

Comments to the second point concerning the housing

The paragraphs "Accomodation and care" are now identical in both descriptions of animal procedures. We also added the following sentence" Related the behavioral task mild water or food restriction will be applied in individual housing."

Comments to the third point concerning the use of males instead of females

We have added to the section "3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed." The following sentence as blue "'Based on these considerations we think that a full study of gender differences, while interesting, is beyond the scope of the current project because it involves studying correlation of neural effect with a new variable, estrogen level, which require a different experimental design."

But see also the following explanation.

Indeed it is commonly accepted that, in the APPswePS1dE9 mouse model, Aβ burden of aged females appears to be more severe than in old males (1-4). However, this does not only apply to aged animals but also younger mice. In 4-month old APPswePS1dE9 mice, brain Aβ load has been reported to be dominated by Aβ1–40 rather than Aβ1–42 (1,2). Upon 6 months of age, Aβ ratio shifts towards Aβ1–42 that is maintained until end of life. Furthermore, a recent study showed that senile plaques in cortex and hippocampus can be detected in 3-month-old males but barely in females of the same age which means that Aβ plaques occur earlier in males (5).

Based on the accumulation of different proportion of amyloid forms in different ages and between genders it has been noted that in the age of 4 month males Abeta40 level is 71.1±2.5 ng/g compared to female 89.9±3.2 ng/g, and the same difference is also seen in Abeta42 level, male 15.4. ±1.8 vs. female 27.5±2.2 (mean±SEM). However, in the age of 12 months Abeta40 levels in male is 1432±512 ng/g and in females 4585±1451 ng/g, and Abeta 42 levels in males 3564±993 ng/g and in females 9566±2249 ng/g (2). However, it has to be noted that even though one of these Abeta levels or the ration of Abeta40/Abeta42 could be likely linked to the observed neuronal hyperactivity, we have shown that amount of epileptic seizures is reduced already in the 15-18 weeks when levels of soluble forms of amyloid still increasing (7). It has to be also noted that we have observed hyperactivity in brain areas which do not show amyloid accumulation (6). To conclude, gender differences in soluble form of amyloid are not reliable predictor of observed neuronal hyperactivity.

To my knowledge, there is currently only one study focusing on the gender difference in amyloid related hyperactivity (8). In this study the researchers focused only to hippocampal activity instead thalamocortical connections which we have shown to be mostly affected in young animals (6). In addition, in this study hyperactivity was only registered as seldom occurring spikes and changes in overall brain oscillations. Also, the findings were mainly compared as wild type vs. transgenic mice both in female and male groups making comparison between genders difficult. Therefore, the results were rather mixed. For instance, spike train duration showed that in the age 14 week male transgenic animals have longer spike train duration than wild type males (0.6±0.3 vs. 0.1±0.01 min). However, there was no difference in female transgenic and wild type animals (0.3±0.3 vs 0), and based on my own analysis of their results there was no difference between genders in this age group (14 weeks). However, already in the age 15 week there is a difference between female wild type mice (0.1±0.01) and transgenic females (0.3±0.1) but not any more in male. My own analysis didn't show any differences between genders. However even though there are no difference between wild type and transgenic animals either in male or female groups it seems that there is a gender difference in age 19 weeks in transgenic animals (male 0.4±0.2 vs. female 1.2 ±0.4). But as said this only applies in spike train duration and not in amount of single spikes which is another main parameter in this experiment while determining the hyperactivity related to amyloid accumulation in different genders.

In summary, it is obvious that there are differences in the amyloid accumulation between the genders in this transgenic animal strain. However, the results correlating gender to amyloid related activity have so far been sparse and rather mixed.

Most likely above mentioned facts are related to development of brain and/or differential expression of estrogen/testosterone effects in the neuronal activity and progression of amyloid pathology. This is noted not only in the epidemiological studies (e.g. 9) but also animal models of amyloid accumulation (e.g.10, 11). Therefore, if we wanted to study the proposed amyloid related hyperactivity also in females we would need separated groups for males and females both of which would have transgenic and control animal groups. Therefore, the total amount of animals would be 770 x2 = 1540. If the estrogen level during amyloid pathology is one of the key factors for hyperactivity we would also need to control the stage of the estrous cycle of the mouse (4 stages) with individual groups making the amount of females needed in the actual experiments (not in breeding) (770x4= 3080 in addition to males 770 (12).

Based on these considerations we think that a full study of gender differences, while interesting, is beyond the scope of the current project because it involves studying correlation of neural effect with a new variable, estrogen level, which require a different experimental design. This is definitely an interesting question, but one that needs a dedicated study, for which new personnel and financial resources need to be acquired.

1. Garzia-Alloza et al. Neurobiology of Disease 2006
2. Wang et al. Neurobiology of disease 2003
3. Ordonez-Gutierrez et al. Journal of Alzheimer's disease 2016
4. Jiao et al. Neurotoxicity Research 2016
5. Ordonez-Gutierrez et al. Journal of Alzheimer's disease 2015
6. Gurevicius et al. Cerebral cortex 2013
7. Minkevience et al Journal of Neurocience 2009
8. Papazoglou et al. Neural plasticity 2016
9. Mielke et al. Clinical Epidemiology 2014
10. Carroll et al. Brain Research 2011
11. Placanica et al. PLoS One 2009.
12. Byers et al. PLoS One 2012

Geachte [redacted]

We hebben vandaag telefonisch contact gehad over enkele onduidelijkheden in uw aanvraag met nummer AVD1030020171327 en titel 'Alzheimer's disease and hyperactivity'. Deze betreffen het hergebruik, de afwijkende huisvesting van de dieren en het gebruik van alleen mannelijke dieren.

1) In de aanvraag staat dat dieren worden hergebruikt in andere gedragstesten. Indien voor deze gedragstesten dezelfde chirurgische ingreep nodig is en de testen onder dezelfde onderzoeksvraag vallen is er geen sprake van hergebruik. Indien een willekeurig ander dier met een andere chirurgische ingreep voor die andere gedragstesten zou kunnen worden gebruikt, dan is er sprake van hergebruik. In dit geval is wettelijk verboden om dieren die in een eerder experiment of in het huidige experiment ernstig ongerief ervaren te hergebruiken.

Artikel 1e,lid 1 van de Wod:
1. Wanneer bij een dierproef ook een dier kan worden gebruikt waarop nog niet eerder een dierproef is verricht, wordt slechts een dierproef verricht met een dier dat al eerder in een dierproef is gebruikt, indien voldaan is aan de volgende voorwaarden:

- **a.** de werkelijke ernst van pijn, lijden, angst of blijvende schade waarmee de voorgaande dierproeven gepaard gingen «licht» of «matig» was;
- **b.** vastgesteld is dat de algemene gezondheids- en welzijnstoestand van het dier volledig is hersteld;
- **c.** de volgende dierproef op basis van artikel 10b is ingedeeld in de categorie: «licht, «matig» dan wel «terminaal»; en
- **d.** de te verrichten handeling in overeenstemming is met diergeneeskundig advies, bij de totstandkoming waarvan rekening wordt gehouden met de volledige levensloop van het dier.

We verzoeken u om duidelijk aan te geven of inderdaad in dit project sprake is van hergebruik. Zo ja, dan mag geen sprake zijn van ernstig ongerief (aangegeven in de huidige versie van de bijlage dierproeven 1). Zo nee, dan graag de bijlages dierproeven aanpassen.

2) In de aanvraag wordt vermeld dat dieren mogelijk individueel worden gehuisvest wegens voedsel- of waterrestrictie en de implantaten. Deze informatie is gedeeltelijk in bijlage dieproeven 2 onder punt F vermeld, maar ontbreekt in bijlage dierproeven 1 onder F. Zou u dit willen aanpassen?

3) U geeft aan alleen mannelijke dieren te willen inzetten omdat er geslacht-specifieke verschillen zijn. De CCD zou graag willen weten hoe groot deze verschillen zijn en tot hoeveel extra dieren zou het gebruik van beide geslachten leiden. We verzoeken u deze informatie bij ons in te dienen.

Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. Om uw aanvraag in de eerstkomende CCD vergadering te kunnen behandelen ontvangen we uw reactie graag uiterlijk **donderdag, 20 april 2017.**

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

Met vriendelijke groet,

[redacted]
Uitvoeringsexpert

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

10.

[redacted]

Van: [redacted]
Verzonden: dinsdag 25 april 2017 14:01
Aan: info@zbo-ccd.nl
Onderwerp: FW: NTS aanvraag AVD1030020171327
Bijlagen: NTS AVD1030020171327 vs 2.pdf

Beste [redacted]
Nadat ik de vorige versie had verstuurd kreeg ik nog een reactie van de onderzoeker, dat de aantallen in sectie 3.3 correct waren, ipv de aantallen in sectie 3.5. Ik heb dit nu aangepast. Hierbij de laatste versie, en mijn excuses voor de verwarring.
Hartelijke groet,
[redacted]

Van: [redacted]
Verzonden: dinsdag 25 april 2017 13:38
Aan: 'Info-zbo'
Onderwerp: RE: NTS aanvraag AVD1030020171327
Beste [redacted]
Excuus, hierbij de nieuwe versie!
Hartelijke groet,
[redacted]

Van: Info-zbo [mailto:info@zbo-ccd.nl]
Verzonden: dinsdag 25 april 2017 13:34
Aan: [redacted]
CC: [redacted]
Onderwerp: RE: NTS aanvraag AVD1030020171327
Beste [redacted]
Hartelijk dank voor de NTS. Het punt is dat nu verschillende percentages onder 3.3 en 3.5 staan.
Omdat bij punt 3.3 wordt naar de diersoorten en aantallen gevraagd, zouden jullie kunnen overwegen om daarin alleen deze informatie te laten en het ongerief en bijhorende percentages onder 3.5 te zetten. Anders graag dezelfde percentages in beide vakjes houden, dubbele tekst dus.
Ik ontvang graag de aangepaste NTS.
Met vriendelijke groet,

[redacted]
Uitvoeringsexpert
Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

Van: [redacted]
Verzonden: dinsdag 25 april 2017 13:14
Aan: info@zbo-ccd.nl
Onderwerp: RE: NTS aanvraag AVD1030020171327
Beste [redacted]
Dank u wel voor de snelle reactie. Hierbij de aangepaste NTS. We zien de beschikking graag tegemoet.
Hartelijke groet,
[redacted]

Van: Info-zbo [mailto:info@zbo-ccd.nl]
Verzonden: dinsdag 25 april 2017 13:06
Aan: [redacted]
Onderwerp: RE: NTS aanvraag AVD1030020171327
Beste [redacted]
De NTS kan ook per email worden gestuurd, maar als jullie het liever via de NetFTP willen sturen is morgen ook goed.
De CCD heeft de aanvraag besproken, maar de beschikking kan niet naar jullie toe worden gestuurd voordat we de nieuwe NTS hebben ontvangen.
Met vriendelijke groet,

[redacted]
Uitvoeringsexpert

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

Van: [redacted]
Verzonden: dinsdag 25 april 2017 12:11
Aan: info@zbo-ccd.nl
Onderwerp: RE: NTS aanvraag AVD1030020171327

Beste [redacted]
Dank u wel voor de mail en het attenderen op deze fout in de NTS. We hebben het gewijzigd, maar ondervinden problemen bij het uploaden. Bij documenten die onze secretaresse eerder vandaag probeerde te uploaden kregen we al een foutmelding, nu kan ze überhaupt niet meer uploaden. Is het een probleem als de aangepaste NTS morgen jullie kant op komt, of zal ik deze via mail aan jullie sturen?

Hartelijke groet,

[redacted]
www.radboudumc.nl

Van: Info-zbo [mailto:info@zbo-ccd.nl]
Verzonden: dinsdag 25 april 2017 9:31
Aan: [redacted]
CC: [redacted]
Onderwerp: NTS aanvraag AVD1030020171327

Geachte [redacted]
In de NTS horend bij de aanvraag AVD1030020171327 staat onder punt 3.3 dat dieren licht, matig of ernstig ongerief ervaren, maar onder punt 3.5 staat dat 'Het ongerief zal matig zijn voor alle dieren.'
Zou u dit willen aanpassen en een nieuwe NTS zo snel mogelijk naar ons toe sturen?
De behandeling van uw aanvraag wordt opgeschort totdat we de nieuwe NTS hebben ontvangen.
Alvast hartelijk dank.
Met vriendelijke groet,

[redacted]
Uitvoeringsexpert
Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

Het Radboudumc staat geregistreerd bij de Kamer van Koophandel in het handelsregister onder nummer 41055629.
The Radboud university medical center is listed in the Commercial Register of the Chamber of Commerce under file number 41055629.

Het Radboudumc staat geregistreerd bij de Kamer van Koophandel in het handelsregister onder nummer 41055629.
The Radboud university medical center is listed in the Commercial Register of the Chamber of Commerce under file number 41055629.

11.

[redacted]

Van: [redacted]

Verzonden: woensdag 19 april 2017 11:46
Aan: info@zbo-ccd.nl
Onderwerp: RE: aanvullende informatie aanvraag AVD1030020171327

Categorieën: Dossier: Andreea

Beste [redacted]

In overleg met de voorzitter van de RUDEC kom ik tot het volgende antwoord op uw vraag.

In de EU-richtlijn staat in de Algemene Bepalingen Artikel 33 (**Verzorging en huisvesting)**:

1. Wat betreft de verzorging en de huisvesting van de dieren dragen de lidstaten er zorg voor dat:

a) alle dieren huisvesting, een omgeving, voedsel, water en verzorging ontvangen die passend zijn voor hun gezondheid en welzijn;

b) iedere inperking van de mogelijkheid van de dieren om aan hun fysiologische en ethologische behoeften te voldoen, tot een minimum wordt beperkt;

c) de omgevingsomstandigheden waarin de dieren worden gefokt, gehouden of gebruikt, dagelijks worden gecontroleerd;

d) regelingen worden getroffen om een eventueel letsel of pijn, onnodig lijden, angst en blijvende schade die vermijdbaar zijn en die worden ontdekt, zo snel mogelijk te verhelpen, en

e) de dieren onder behoorlijke omstandigheden worden vervoerd.

2. Met het oog op het bepaalde in lid 1, zorgen de lidstaten ervoor dat de in bijlage III omschreven verzorgings- en huisvestingsnormen worden toegepast met ingang van de in die bijlage gespecificeerde data.NL 20.10.2010 Publicatieblad van de Europese Unie L 276/
3. De lidstaten kunnen om wetenschappelijke redenen, of redenen van dierenwelzijn of diergezondheid afwijkingen van lid 1, onder a), en lid 2 toestaan.

Uit punt 3 blijkt dat om wetenschappelijke reden of redenen van dierenwelzijn afgeweken kan worden van 'normale' huisvesting. De individuele huisvesting van dieren om te voorkomen dat hun headpost beschadigd wordt door een kooigenoot en om voedselinname te kunnen controleren is in onze ogen dus niet afwijkend van hetgeen in de richtlijn staat.

In bijlage III wordt hier ook naar verwezen: Met uitzondering van de soorten die van nature solitair zijn, moeten dieren in sociaal verband worden gehuisvest in stabiele groepen van compatibele individuen. In gevallen van afzonderlijke huisvesting op grond van artikel 33, lid 3, moet de duur van de afzondering tot het noodzakelijke minimum worden beperkt en moet het visuele, auditieve, olfactorische en/of tactiele contact worden gehandhaafd. De introductie of herintroductie van dieren in bestaande groepen moet zorgvuldig in het oog worden gehouden, teneinde problemen als gevolg van onverenigbaarheid of verstoorde sociale relaties te vermijden.

Dieren die individueel gehuisvest zijn hebben wel visueel, auditief en olfactorisch contact. Hierdoor is de individuele huisvesting van de dieren volgens de RUDEC conform de eisen in bijlage III van de richtlijn.

Graag hoor ik of de CCD het eens is met deze redenatie.

Onlangs heeft u dezelfde vraag gesteld over een advies dat de RUDEC heeft uitgebracht voor een andere projectaanvraag (AVD103002017872). Daar heeft u op 9-3-2017 een vergelijkbaar antwoord opgekregen (inclusief het verzoek om feedback), maar wij hebben daar geen feedback over ontvangen. Deze zouden wij op prijs stellen, mede gezien het feit dat u dezelfde vraag nogmaals stelt.

Vriendelijke groeten,
[redacted]

Van: Info-zbo [mailto:info@zbo-ccd.nl]
Verzonden: donderdag 13 april 2017 17:49
Aan: [redacted]
Onderwerp: aanvullende informatie aanvraag AVD1030020171327

Geachte RUDEC,

Op 6 april 2017 heeft u advies uitgebracht op een projectaanvraag met titel 'Alzheimer's disease and hyperactivity' en nummer AVD1030020171327.

In uw advies bij vraag C10 geeft u aan dat de huisvesting van de dieren volgens bijlage III van de richtlijn is. De aanvrager meldt in de aanvraag dat de dieren mogelijk individueel worden gehuisvest. Hoewel de reden hiervoor onderbouwd is, wijkt de huisvesting van eisen in bijlage III af. We zouden graag willen weten hoe u hiernaar heeft gekeken.

We ontvangen uw reactie graag uiterlijk donderdag 20 april 2017.

De volgende vragen zijn aan de aanvrager voorgelegd:

1) In de aanvraag staat dat dieren worden hergebruikt in andere gedragstesten. Indien voor deze gedragstesten dezelfde chirurgische ingreep nodig is en de testen onder dezelfde onderzoeksvraag vallen is er geen sprake van hergebruik. Indien een willekeurig ander dier met een andere chirurgische ingreep voor die andere gedragstesten zou kunnen worden gebruikt, dan is er sprake van hergebruik. In dit geval is wettelijk verboden om dieren die in een eerder experiment of in het huidige experiment ernstig ongerief ervaren te hergebruiken.

Artikel 1e, lid 1 van de Wod:
1. Wanneer bij een dierproef ook een dier kan worden gebruikt waarop nog niet eerder een dierproef is verricht, wordt slechts een dierproef verricht met een dier dat al eerder in een dierproef is gebruikt, indien voldaan is aan de volgende voorwaarden:

- **a.** de werkelijke ernst van pijn, lijden, angst of blijvende schade waarmee de voorgaande dierproeven gepaard gingen «licht» of «matig» was;
- **b.** vastgesteld is dat de algemene gezondheids- en welzijnstoestand van het dier volledig is hersteld;
- **c.** de volgende dierproef op basis van artikel 10b is ingedeeld in de categorie: «licht, «matig» danw el «terminaal»; en
- **d.** de te verrichten handeling in overeenstemming is met diergeneeskundig advies, bij de totstandkoming waarvan rekening wordt gehouden met de volledige levensloop van het dier.

We verzoeken u om duidelijk aan te geven of inderdaad in dit project sprake is van hergebruik. Zo ja, dan mag geen sprake zijn van ernstig ongerief (aangegeven in de huidige versie van de bijlage dierproeven 1). Zo nee, dan graag de bijlages dierproeven aanpassen.

2) In de aanvraag wordt vermeld dat dieren mogelijk individueel worden gehuisvest wegens voedsel- of waterrestrictie en de implantaten. Deze informatie is gedeeltelijk in bijlage dieproeven 2 onder punt F vermeld, maar ontbreekt in bijlage dierproeven 1 onder F. Zou u dit willen aanpassen?

3) U geeft aan alleen mannelijke dieren te willen inzetten omdat er geslacht-specifieke verschillen zijn. De CCD zou graag willen weten hoe groot deze verschillen zijn en tot hoeveel extra dieren zou het gebruik van beide geslachten leiden. We verzoeken u deze informatie bij ons in te dienen.

Indien u ook hierop wil reageren horen we het graag uiterlijk donderdag 20 april 2017.

Met vriendelijke groet,

[redacted]
Uitvoeringsexpert

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

Het Radboudumc staat geregistreerd bij de Kamer van Koophandel in het handelsregister onder nummer 41055629.
The Radboud university medical center is listed in the Commercial Register of the Chamber of Commerce under file number 41055629.

13.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen
[redacted]
Postbus 9101
6500 HB NIJMEGEN

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

Onze referentie
Aanvraagnummer
AVD1030020171327
Bijlagen
1

Datum 25 april 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 7 april 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Alzheimer's disease and hyperactivity" met aanvraagnummer AVD1030020171327. Wij hebben uw aanvraag beoordeeld.

Op 20 en 24 april 2017 heeft u uw aanvraag aangevuld. De vragen van de CCD over hergebruik, huisvesting en het geslacht van de te gebruiken dieren zijn beantwoord. De aanvraag is ook aangepast.

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

U kunt met uw project "Alzheimer's disease and hyperactivity" starten. De vergunning wordt afgegeven van 8 mei 2017 tot en met 7 mei 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.
Er is sprake van ongerieffcategorie Ernstig.

Datum:
25 april 2017
Aanvraagnummer:
AVD1030020171327

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie RU DEC gevoegd. Dit advies is opgesteld op 6 april 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 19 april 2017 heeft de DEC gereageerd op onze vragen. De DEC heeft de vraag van de CCD over de huisvesting beantwoord.
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]

Algemeen Secretaris

Datum:
25 april 2017
Aanvraagnummer:
AVD1030020171327

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:	Stichting Katholieke Universiteit Nijmegen
Adres:	Postbus 9101
Postcode en plaats:	6500 HB NIJMEGEN
Deelnemersnummer:	10300

deze projectvergunning voor het tijdvak 8 mei 2017 tot en met 7 mei 2022, voor het project "Alzheimer's disease and hyperactivity" met aanvraagnummer AVD1030020171327, volgens advies van Dierexperimentencommissie RU DEC. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is [redacted] Voor de uitvoering van het project is Instantievoor Dierenwelzijn verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 7 april 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 20 april 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 25 april 2017;
c Advies van dierexperimentencommissie d.d. 6 april 2017, ontvangen op 7 april 2017.
d De aanvullingen op uw aanvraag, ontvangen op 20 en 24 april 2017

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 Circuit manipulations in behaving, head-fixed mice				
	Muizen (Mus musculus) / wildtype en trangene APPswe en PS1dE9 (APdE9) muislijnen	310	23% Ernstig 45% Matig 32% Licht	
3.4.4.2 Circuit manipulation in freely moving mice				
	Muizen (Mus musculus) / gelijk aan 3.4.4.1	860	33% Ernstig 32% Matig 35% Licht	

Aanvraagnummer:
AVD1030020171327

Voorwaarden
Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen
In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van beoordeling achteraf.Deze beoordeling zal uiterlijk mei 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.

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.

Aanvraagnummer:
AVD1030020171327

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.

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.