		Inventaris Wob-verzoek W17-08								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 2017873**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Origineel aanvraagformulier				x		x	x	
2		NTS oud			x					
3		Projectvoorstel			x					
4		Bijlage oud				x			x	
5		Ontvangstbevestiging en factuur				x		x	x	
6		Verzoek om aanvullende informatie				x		x	x	
7		Antwoord op verzoek om aanvullende informatie			x					
8		Bijlage nieuw				x			x	
9		NTS nieuw	x							
10		DEC advies				x		x	x	
11		Advies CCD		x						x
12		Beschikking en vergunning				x		x	x	

AVD 103002017873

1.

22 FEB. 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 10
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	☑ Dhr. ☐ Mw.
Functie	
Afdeling	
Telefoonnummer	
E-mailadres	

1.6 *(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.

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

1.7 Is er voor deze projectaanvraag een gemachtigde?

☑ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*

☐ Nee

2 Over uw aanvraag

2.1 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 3

☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn

Vul uw vergunde projectnummer in en ga verder met vraag 2.2

☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Vul uw vergunde projectnummer in en ga verder met vraag 2.3

2.2 Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?

☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier

☑ Nee > Ga verder met vraag 3

2.3 Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?

☐ Nee > Ga verder met vraag 3

☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1 Wat is de geplande start- en einddatum van het project?	Startdatum: 17-03-2017
	Einddatum: 17-03-2022
3.2 Wat is de titel van het project?	Interneurons, oscillations, and learning
3.3 Wat is de titel van de niet-technische samenvatting?	Neuronen, oscillaties, en leerprocessen

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 € 1.035,00 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	[redacted]
Plaats	Nijmegen
Datum	17-02-2017
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	Neuronen, oscillaties, en leerprocessen
1.2	Looptijd van het project	17-3-2017 - 17-3-2022
1.3	Trefwoorden (maximaal 5)	Hersenen, leren, beloningen, oscillaties, neuronale communicatie

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

[X] Fundamenteel onderzoek
[] Translationeel of toegepast onderzoek
[] Wettelijk vereist onderzoek of routinematige productie
[] Onderzoek ter bescherming van het milieu in het belang van de gezondheid 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)	De executieve functies (EF), zoals gedragsaanpassingen, het werkgeheugen, en besluitvormingsprocessen, zijn belangrijk voor het functioneren in de samenleving. Verstoringen van de EF zijn een belangrijk onderdeel van hersenziekten zoals Parkinson, ernstige depressie, en schizofrenie. Het meeste onderzoek naar de EF is in mensen gedaan. In mensen kan de gemiddelde activiteit van miljoenen hersencellen tegelijk gemeten worden. Uit onderzoek bleek dat zogenoemde "elektrische hersengolven" een teken zijn van EF. Maar wat precies zijn die hersengolven en wat doen ze voor de communicatie tussen hersengebieden die belangrijk voor EF zijn? De bedoeling van ons onderzoek is meer inzicht vergaren in de neurale mechanismen van de EF en de manier waarop zij betrokken zijn bij leerprocessen en het nemen van beslissingen. Wij gebruiken daarvoor muismodellen. Hierdoor kunnen wij elektroden in de hersenen plaatsen en de activiteit van individuele hersencellen, groepen van hersencellen, en populatie activiteit tegelijkertijd meten. Daardoor kunnen wij de processen van de EF nauwkeuriger in kaart brengen en begrijpen.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	Er zijn vele fundamentele vragen over hoe informatie wordt verwerkt binnen het "EF netwerk" zoals: hoe wordt informatie gedeeld binnen deze regionen, hoe beïnvloedt het EF netwerk processen in sensorische gebieden, wat is de rol van verschillende soorten neuronen hierin, en op welke manier beïnvloedt het EF netwerk bepaalde leerprocessen? Dit fundamentele onderzoek is van belang om in de toekomst beter te kunnen begrijpen wat er in de hersenen mis kan gaan, zoals bijvoorbeeld bij de ziekte van Parkinson. Wij zullen ook al onze data beschikbaar maken aan de wetenschappelijke gemeenschap, waardoor collega's deze data kunnen gebruiken voor het beantwoorden van hun eigen onderzoeksvragen. De resultaten van ons onderzoek kunnen gebruikt worden voor onderzoek naar cognitie en om computermodellen te maken waarmee bepaalde aspecten van de hersenfunctie onderzocht kunnen worden.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	Maximaal 395 muizen over 5 jaar.
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	Om goede metingen te kunnen verrichten worden er speciale elektroden in het brein geplaatst middels een operatie. Deze operaties en het bijkomen uit narcose zullen tot tijdelijk ongerief leiden. Anesthesie, pijnstilling en antibiotica worden gebruikt om dit tot een minimum te beperken. Wij gebruiken lichte water- en voedselbeperkingen om de gedragsmotivatie van de muizen te verhogen. Zij krijgen sap en andere beloningen tijdens de experimenten. Tijdens de metingen mogen de dieren hun kop niet bewegen. De kamer die zij op hun kop dragen om de elektroden te beschermen wordt daarom vastgezet gedurende maximaal 90 minuten per dag. De dieren wennen hier geleidelijk aan. Als tijdens de metingen blijkt dat een dier teveel stress heeft dan worden de metingen gestopt en kan het weer vrij bewegen. De dieren kunnen vanwege de kamer op hun kop niet met soortgenoten in één kooi verblijven.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Alle dieren ondergaan vergelijkbare handelingen die een matig ongerief als gevolg hebben.

3.6	Wat is de bestemming van de dieren na afloop?	Alle dieren worden na afloop van de proef gedood (door middel van anesthesie pijnloos) om hersenweefsel te onderzoeken.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Metingen in mensen zijn niet precies genoeg om zenuwcellen te meten. Daarom zijn dierproeven die hier beschreven worden nog noodzakelijk. De resultaten kunnen gebruikt worden om betere computermodellen te maken waardoor sommige onderzoeksvragen in de toekomst zonder proefdieren kunnen worden beantwoord.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	Wij gebruiken speciale en nieuwe elektroden die heel veel gegevens kunnen verzamelen per dier, waardoor we minder dieren nodig hebben om onze onderzoeksvraag te beantwoorden. Wij maken onze meetgegevens openbaar beschikbaar aan de wetenschappelijke gemeenschap. Andere wetenschappers zullen daardoor minder dierproeven hoeven te doen. We zullen het kleinst aantal dieren gebruiken waarmee nog wetenschappelijk betrouwbare resultaten zijn te behalen.
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.	Wij hebben ervoor gekozen om de hersenen van muizen te onderzoeken, omdat deze dieren over executieve functies beschikken die voldoende lijken op de executieve functies van mensen. Bij minder complexe diersoorten is dat niet het geval. De experimenten en diermodellen die we gebruiken staan bekend om de betrouwbare resultaten. Wanneer mogelijk werken wij aan het verbeteren van onze experimenten en procedures. Het is onze overtuiging dat de beste resultaten behaald worden met gezonde dieren met een zo laag mogelijk stressniveau. We doen er dan ook alles aan om de stress voor de dieren te verminderen, bijvoorbeeld door kleine en lichte elektroden te maken met 3D printing.
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 handelingen zullen worden verricht door ervaren personeel. Alle dieren krijgen de tijd om te wennen aan de nieuwe leefomgeving en de onderzoeker. Muizen moeten alleen gehuisvest worden, maar ze zullen regelmatig onder toezicht contact hebben met soortgenoten. Rondom operaties zal adequate pijnstilling worden gegeven. Rondom operaties wordt nutriëntenrijk voedsel aangeboden om het herstel te bevorderen. In alle gedragsproeven worden muizen beloond (sap, yoghurt, water, enz.). De dieren krijgen nooit vervelende of pijn prikkels.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	

3.

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.	Interneurons, oscillations, and learning

2 Categories

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

[X] Basic Research
[] Translational or applied research
[] Regulatory use 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.

EXECUTIVE FUNCTIONS AND THEIR IMPAIRMENTS

Executive functioning refers to the ability to monitor the environment for mistakes, conflicts, or negative performance feedback, and to initiate rapid but flexible action adjustments to optimize goal-directed behavior (Botvinick et al., 2001; Ridderinkhof et al., 2004). Stimulus-reward association learning is an important aspect of executive functioning, and is amenable to careful neuroscientific investigation because all mammals (including mice, the species under investigation in this research) are capable of forming new stimulus-reward associations. Therefore, fundamental insights into the organization of the executive functioning network can be obtained by studying this network during learning, as well as during spontaneous activity.

Impairments in executive functions are seen in several clinical disorders including major depression and schizophrenia. Understanding the neural circuitry underlying executive functioning may lead to a significant advance in understanding disorders of executive functioning, as well as studying the effects of treatments. In this research, we will focus on the role of subclasses of neurons in regulating rhythmic neural activity and inter-regional synchronization, and how those processes are related to reward learning.

NEURAL OSCILLATIONS IN CIRCUITS UNDERLYING EXECUTIVE FUNCTIONS

Executive functioning relies on a network of inter-connected brain regions, mainly including the prefrontal cortex, parietal cortex, and striatum/basal ganglia (for convenience, this collection of systems will be referred to as the "executive functioning network"). For example, lesions to the medial prefrontal cortex of rats leads to impairments in decision-making and increased impulsivity (Courtière et al., 2007; Walton et al., 2002). Neural oscillations -- rhythmic fluctuations in the excitability of populations of neurons -- are important markers of neural activity. Neural oscillations can be measured through the local field potential -- very thin electrodes placed inside the brain -- and through the electroencephalography (EEG) -- larger electrodes placed on the skull (on the scalp in humans).

Theta-band (4-8 Hz) oscillations in the medial prefrontal cortex are strongly associated with executive functioning in humans (see Cohen 2014, for a review). It has been speculated that theta oscillations are used to synchronize brain activity across regions within the executive functioning network, and between this network and sensory processing areas. In other words, it is thought that neural oscillations are crucial for executive functioning,

and that understanding the role of neural oscillations in executive functioning will lead to advances in understanding healthy executive functioning and impairments in executive functioning in clinical disorders. However, the precise role of oscillations is very difficult to understand using non-invasive methods in humans. Therefore, rodent models are necessary.

ROLES OF INTERNEURONS IN NEURAL OSCILLATIONS AND EXECUTIVE FUNCTIONS

There are thousands of different types of cells in the brain. However, neurons can be broadly classified as excitatory or inhibitory. Excitatory (glutamateric) neurons comprise approximately 75% of the neurons, while inhibitory interneurons are in the minority. However, research is increasingly demonstrating that inhibitory interneurons play key roles in regulating network activity and oscillations, and neural computations (Markram et al., 2004). Furthermore, interneurons can be categorized into different groups according to their molecular signatures, which in turn defines their functional/anatomical properties. In this research, we will focus on four specific subclasses of neurons:

Parvalbumin-positive interneurons (PVB): These cells target primarily perisomatic regions of pyramidal cells and are implicated in high-frequency gamma-band oscillations (Hu et al., 2014).

Somatostatin-positive interneurons (SOM): These cells target primarily dendritic arbors and are implicated in lower-frequency theta/alpha oscillations (Urban-Ciecko and Barth, 2016).

Vasoactive intestinal polypeptide-containing interneurons (VIP): These cells target primarily other interneurons (Pi et al., 2013). VIP cells seem to have an important role in disinhibition, in other words, blocking the inhibition. However, they have not been extensively characterized in terms of their roles in neural functioning and network dynamics.

Pyramidal cells: Interneurons, though important, are a minority of the cells in the brain; most brain cells are pyramidals. Pyramidal cells are thought to be the computational powerhouse of the brain, encoding and transmitting important information about the sensory environment and motor output plans. It is important to include pyramidal cells in the study of interneurons to disambiguate general effects of neural activity from subclass-specific effects.

Relatively little is known about the roles of different interneuron types in oscillations and inter-regional synchronization in the executive functioning network. To make matters more complicated, most of the research on these interneurons is done in cell cultures or in primary sensory regions. Their contributions to regulating activity within the executive network are likely to be at least somewhat different from their roles in primary sensory regions. For example, although PVB cells are strongly implicated in gamma oscillations in visual cortex, PVB cell density is lower in the medial prefrontal cortex (Dombrowski et al., 2001), and the medial prefrontal cortex is dominated by lower-frequency theta oscillations, in contrast to primary sensory regions. Furthermore, short-term plasticity characteristics differ between medial prefrontal and sensory areas (Wang et al., 2006). In other words, although there are certainly features of neural dynamics that are conserved across the brain, there are also region-specific features that require detailed investigations of each brain region.

INTERNEURONS, OSCILLATIONS, AND LEARNING

Learning and decision-making are among the most important executive functions, and are hallmarks of impairments in many psychiatric disorders. It is known that neural oscillations and inter-regional synchronization are involved in stimulus-reward learning (Pennartz et al., 2011). Much less is known about the roles of interneuron classes on reward learning. Most of the existing work on motivated learning concerns fear conditioning and cocaine addiction (Nieh et al., 2013). Certainly these are important and clinically relevant topics of investigation. But a considerable amount of real-life learning involves simpler and less dramatic learning processes (e.g., reward-based learning) that require integration across cortical, striatal, and sensory-processing areas. Therefore, we will focus on simple stimulus-reward learning as a model to understand the role of neuron subtypes in synchronizing brain circuits during learning. During stimulus-reward learning, network-level neural synchronization is increased between the medial prefrontal cortex, striatum, and sensory processing regions. Our research will focus on the roles of different neuron subclasses in changes in these

patterns neural synchronization that occur during learning. This will be achieved by recording neural activity before, during, and after learning, as well as by interrupting activity in specific types of neurons during the learning process.
Our research involves focusing both on fundamental mechanisms of brain network dynamics, and on understanding how these fundamental mechanisms are involved in sensory processing and stimulus-reward association learning.
Note that learning is not the only situation associated with oscillations. Neural oscillations are present in many different behavioral/cognitive states, including running, memory, and even sleep. We focus on learning in this research because it allows us to link our findings to theories and data from human about the roles of neural oscillations in learning. Because oscillations are a fundamental feature of brain activity, it is likely that many aspects of our findings are also applicable to other cognitive/behavioral states. Learning per se has been studied extensively over the past century. We will use well-established basic learning experiment paradigms that allow us to link our novel findings (roles of interneurons in synchronization) to the established literature, including the literature in humans.

OSCILLATIONS IN EEG: WHAT DO THEY MEAN?
In humans, EEG is the primary method for studying the electrical activity of the brain. Oscillations are one of the most prominent features in the EEG signal. However, in humans it is not possible to link the EEG signal to lower-level neural activity. Thus, an important implication of our research is to help understand what happens in the brain when different EEG signals are measured. To answer this question, all animals will have EEG recorded from the skull along with recordings of lower-level neural activity (neurons and local field potential).
WHY WE ARE DOING THIS RESEARCH
The overall goal of the research in our group is to understand the roles of neural oscillations in executive functioning. We have several ongoing projects that span humans, computational models, rats, and mice. Each method has its advantages. The primary advantage of mice is the ability to use cutting-edge genetic tools to target specific classes of neurons (focusing on PVB, SOM, VIP, and pyramidals). These mouse lines in combination with optogenetics allow us to manipulate the activity of these subclasses. Mice provide a good balance between molecular specificity and cognitive/neural development. Thus, this project is a core component of our lab's ongoing research.
The result of this research will be a better understanding of how different types of neurons contribute to stimulus-reward association learning. This will be applicable to basic research in brain function and executive control, as well as interpreting patterns of results seen in human EEG studies. By incorporating neural stimulation, this research also has important translational and application value. For example, brain stimulation techniques (transcranial magnetic and electrical stimulation) are increasingly being applied as a treatment option in humans for Parkinson's disease and major depression, among other disorder. Our research will help understand how these manipulations affect neural activity in the brain systems most important for executive functioning.
Another important component of this research is the application and development of cutting-edge data analysis techniques for characterizing neural activity. We will also make our data available (at data.donders.ru.nl, a university-supported infrastructure for storing, managing, and public sharing of research data) to other scientists for further development and analysis. In this way, the data will be used beyond our research, which facilitates the Replacement and Reduction principles of animal ethics. That is, other researchers will be able to use our data rather than acquiring new data in new animals.

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Walton ME, Bannerman DM, Rushworth MF. (2002) The role of rat medial frontal cortex in effort-based decision making. J Neurosci.;22(24):10996-1003.

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 primary purposes of this research are:

(1) Determine the roles of three interneuron classes (PVB, SOM, VIP) and pyramidal cells on local neural oscillations in the executive functioning network, and on long-range information transfer between sensory processing regions and the executive functioning network. This goal will be achieved by applying brief pulses of electrical microstimulation pulses to one region while simultaneously measuring neural activity from the same region and from other regions. Furthermore, we will use optogenetics to identify and silence/activate different neuron classes while measuring neural activity using electrophysiology and calcium imaging (for this purpose, small electrodes or imaging lenses will be placed in the executive functioning network including the prefrontal cortex and striatum, and sensory-processing regions including V1 and S1). Recordings will take place during learning and spontaneous ("resting-state") activity. We will use different temporal patterns of microstimulation (varying from single pulses to 100 Hz) to determine frequency resonance responses while using optogenetics to suppress the different neuron classes. We expect to find region- and neuron subtype-specific coupling. The frequency specificity of the results will provide important information into the speed with which information can be transferred across different brain circuits, and how information transfer at different frequencies depends on specific types of interneurons.

(2) Determine the roles of these neuron classes in learning and decision-making in the executive functioning network. This goal will be achieved by having mice learn that some sensory stimuli are associated with rewards while others are not, while neuron classes are optogenetically inhibited or phasically activated during or after stimulus presentation. Brain activity will be monitored simultaneously using electrophysiology or calcium imaging. Neural activity will be recorded continuously, across different phases of learning (before and after learning has taken place), which will allow us to identify the specific roles of neuron classes in learning-related changes in neural activity and inter-regional synchronization. Furthermore, we will use novel stimuli when animals have learned the correct stimulus-reward associations, which will allow us to have a sufficient number of repetitions (trials) to study changes in neural activity during the learning process. We expect to find that synchronization between sensory-processing regions and the executive function network will enhance with learning, and will depend on specific interneuron subtypes. For example, we expect SOM cells to be more involved in slower synchronization while PVB cells are more involved in faster synchronization. For this goal, it is also important to test learning in different sensory modalities (visual, auditory, whisker) in order to determine the modality-specific vs. modality-general features of synchronization with the executive functioning network.

Feasibility of the research

This research is highly feasible. We will utilize neuroscience techniques that have been developed over the past decade and that are currently being used at our research institute. The novelty and importance of this research comes from the innovative application of existing techniques to answer unknown questions. Furthermore, the research is developed with the 3 R's in mind; sophisticated experimental techniques will reduce discomfort of the animals while simultaneously increasing the amount of data obtained from each animal.

The research proposed here will be completed by a team of scientists who have previous experience using electrophysiology and optogenetics. Over the next 5 years, there will be several experienced scientists involved in the research, including 3-4 postdocs, 1-2 PhD students, and a full-time animal technician with 9 years experience. The proposed research will also benefit from close collaborations within the Radboud/Donders community. The PI has also written an authoritative textbook on neuroscience data analyses and statistics.

A large portion of this research is funded by an ERC Starting grant. The purpose of the grant is to make new discoveries regarding the neurobiological origin and functional significance of neural oscillations in the medial prefrontal cortex during executive functioning, and their relationship to EEG signals. This research is also supported by internal funds from the University Medical Center and by NWO grants (e.g., Veni).

3.3 Relevance

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

Scientific relevance:

There is overwhelming evidence that the executive functioning network is critically important for healthy brain function and for learning associations between sensory information and rewards. But there is too little understanding of the microcircuit dynamics that support these aspects of cognition, in particular, the roles of different molecularly-identifiable classes of neurons that control neural oscillations involved in executive functioning. The findings obtained in this research will be significant for researchers of human EEG and cognition, and for researchers of animals and computational models. A second scientific impact is that >10 TB of rich, high-quality neurophysiology data will be available to the scientific community, maximizing the knowledge gained from our data, and thus also reducing the number of animals that need to be used to obtain such data in the future.

Clinical relevance:

Dysfunction of executive control is implicated in many clinical disorders, ranging from mood disorders (obsessive compulsive disorder, major depression) to motor diseases (Parkinson's disease) to schizophrenia and addiction. It is known that these conditions are associated with aberrant patterns of neural activity and synchronization, but it is less clear what those patterns mean at the neural and circuit level, due to limitations of non-invasive neuroimaging in humans.

On the other hand, medications that are associated with improved executive functioning also alter patterns of neural synchronization. Using rodent models to understand basic brain functioning in the executive network will provide a foundation for understanding how these networks are affected by disease models, and how efficacious medication improves functioning in these neural circuits.

Societal relevance:
Executive functioning is broadly relevant for many aspects of success in our world, ranging from social situations to work promotions to driving a car. The research described here will help understand how these functions are implemented in the brain, which is a necessary step towards understanding how these dysregulation in executive functioning contributes to neural disorders. In summary, the scientific, clinical, and societal significance of our research outweighs the potential discomfort the animals may experience.

3.4 Research Strategy

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

Our research strategy involves the following three phases, with associated go-nogo decision points:

1. Surgeries to implant electrodes and inject viruses. Surgery must take place over two sessions due to limitations of the viral injection in the biosafety hood. The first surgery will involve injecting viruses and will last 1-4 hours. This surgery is necessary to allow us to control the activity of subclasses of neurons. This will provide the key experiment feature that allows us to establish causal relationships between neuron types, neural synchronization, and EEG.

The second surgery will take place 1-3 weeks later, will involve implanting electrodes and/or optimal imaging lens, and will last 5-9 hours, depending on the complexity of the surgery. We use state-of-the-art electrodes that maximize the amount of data while minimizing the size of the probes. Each probe is 15-50 microns thick, is very flexible, and is lowered into the brain slowly. These features minimize tissue damage. The lens for optical imaging is placed on top of the brain, under the skull. Some pressure against brain tissue is required to ensure a tight fit with minimal possibility of infection. For this reason, the lens cannot be placed on top of blood vessels.

Go-nogo decision point: Not all animals can be implanted with a lens for optical imaging. Approximately 40% of mice have blood vessel organization under the skull that prevents the safe implantation of a lens (see also DAP, section 2A). Initial screening is performed to examine local vasculature (following established procedures by Low et al., 2014); animals that have veins overlying target brain regions will receive electrodes and not imaging lenses. (Electrode implantation requires only very small burrholes in the skull and it is easy to avoid blood vessels.)

2. Recording sessions that involve brief pulses of electrical or optogenetic microstimulation, during continuous monitoring of brain electrical and neural activity using electrodes and/or optical imaging. This second part lasts no longer than 10 months or shorter if humane endpoints are reached. This part of the research will provide the data that will be analyzed off-line. The analyses are described in more detail in the Animal Procedures section. Briefly, we will focus on identifying the roles of different subclasses of neurons in oscillations and inter-regional synchronization, with a particular focus on the medial prefrontal cortex and its interactions with striatal and sensory-processing regions. Some experiments involve stimulus-reward associations; others involve resting state (no task).

Go-nogo decision point: If animals lose weight or exhibit other signs or disease or discomfort, or if humane endpoints points are reached, the experiment will immediately be stopped (see section H3 for more details). Individual recording sessions will end immediately when there are signs of stress or discomfort, or lack of motivation (e.g., no longer licking for rewards), or after 90 minutes.

3. Euthanasia and ex-vivo anatomical confirmation of electrode implantation and viral expression. This third part ends the research for the animal.

Each animal will undergo the same three procedures in the same order. Differences across animals arise in the specific configuration of electrodes and learning tasks, which are implemented to target different brain regions. However, these specific aspects do not change the overall flow or the strategy of the research, nor do they affect the amount of discomfort. Thus, from an ethics perspective and considering the animals' welfare, the entire research can be considered as one experiment protocol.

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.

Phase 1: Surgery
Surgery is performed to implant electrodes, light fibers, and inject viruses to measure and modulate brain activity. The first surgery takes place in the biosafety cabinet and involves using a human-controlled robotic stereotaxis to inject the virus. Due to space and time constraints of the biosafety cabinet, the second surgery to implant the electrodes/lens will take place in a separate session outside the biosafety cabinet.

Electrodes will be implanted to record neural activity from several distinct neural circuits simultaneously. Depending on the target regions, 1-4 electrodes will be implanted into the brain. In order to establish causal mechanisms, we will also provide brief stimulation using optical or electrical tools. For this purpose, a light fiber is implanted that will be connected to a light source during the experiment. After implantation, the electrodes and fibers are secured to the skull using a combination of skull screws, cement, and other adhesive materials. This allows for chronic recordings for weeks to months, maximizing the amount of data generated per animal and minimizing the total numbers of animals utilized. After recovery, there is no physical pain or discomfort associated with the implantation. We use custom-designed materials that are 3D-printed to be very strong and yet very light.

Phase 2: Tasks and recordings
Adult mice will be tested in different situations during awake head-fixation. The use of chronic non-anesthetized animals is a strong advantage because it minimizes discomfort and produces a very large amount of data from each individual. Animals can be tested up to five days per week for up to 90 minutes per day (less time if there are signs of discomfort). In practice there are typically fewer measurements due to scheduling,

equipment availability, possible delays due to technical difficulties or building renovations, or when the animal is stressed or unmotivated to participate. The maximum will be reached only if the equipment is available and if the animal is sufficiently motivated and healthy. The total number of recording sessions is based on the amount of data required to obtain suitable statistically sound results. Having too little data would prevent us from drawing reliable scientific conclusions. This is described in more detail in a later section. Brain activity is recorded continuously during the testing sessions. Cameras and breathing monitors are available to allow behavior and physiology to be monitored for signs of distress.

Behavioral tasks are similar in that they involve learning stimulus-reward associations and behavioral responses to indicate choices. Differences among tasks are designed to focus on different aspects of learning and associated neural circuitry, and are described in more detail in the Procedures section. Water and/or food scheduling is used to facilitate training and motivation during the behavioral tasks. Frequent liquid rewards are given during the tasks, and ad libitum after recording sessions. We take special care regarding the amount of food/water scheduling -- animals should be motivated to perform the experiments, but they should not be stressed or unhealthy. Healthy animals provide high-quality data, and this is obviously something we want to achieve.

Phase 3: Euthanasia and post-experiment anatomical confirmation
This is necessary to check the anatomical location of the implantations and expression of the virus (for optogenetics).

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 three phases of the research (surgery, recordings, euthanasia) are necessarily highly related. There is no point in doing surgery if there are no tasks to record data, and we cannot record neural activity during behavior without first performing surgery to implant the electrodes. The procedures we will use reflect the current state-of-the-art in neuroscience. We wish to understand the neural circuit mechanisms of executive functioning and control over sensory processing, and large-scale electrophysiology and interference with optogenetic/electrical microstimulation are currently the best tools for this. This is fundamental research that is necessary in order for future research to use rodents as models for studying diseases of human executive functioning.

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	Description of procedures for surgery and tasks.

4.

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 Description of procedures for surgery and tasks.

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.

METHODS TO MEASURE BRAIN ACTIVITY, AND PRIMARY OUTCOME MEASURES

Electrophysiology: In-vivo electrophysiology is a technique that allows us to monitor ongoing brain activity in real-time during awake behavior or sleep. We implant 1-4 very thin probes into the brain that have dozens to hundreds of tiny electrodes. Each electrode acts as an antenna to record the activity of individual brain cells, as well as the local field potential (the aggregated activity over hundreds of cells). Placing electrodes in different parts of the brain also allows us to measure the interactions across brain regions, which are believed to be of central importance to brain functions like perception, memory, and thinking. Primary outcome measures: action potentials from individual neurons, local field potentials

EEG: EEG is one of the primary brain imaging methods in humans. However, EEG measures only aggregated activity summed over millions of neurons. One of the themes of our research is to understand the origin and significance of EEG. Thus, skull EEG will be measured alongside the electrophysiology. From a practical and welfare perspective, EEG adds no discomfort—the setup already requires 4-10 metal skull mounts that secure the headstage; we will simply attach wires to use as electrodes. Primary outcome measure: EEG activity (oscillations in different frequency bands)

Calcium imaging: Calcium imaging is a technique in which a lens is chronically implanted in or on the brain. During recording sessions, the lens is connected to a camera. The camera images fluorescence that is given off by activation of calcium channels, one of the key currents in active neurons. AAV viruses in combination with Cre driver lines provide these fluorescence proteins only to neurons that have a specific molecular marker, such as somatostatin for SOM interneurons. This technique therefore allows us to measure the activity of several dozens of individual cells simultaneously and reliably over time. The use of calcium imaging lenses in the medial prefrontal cortex is well established (Low et al., 2014) and used by many neuroscience research groups in the world, including the Netherlands. Primary outcome measure: activity of cells identified in images

METHODS TO CAUSALLY PROBE BRAIN ACTIVITY

Optogenetics: "Optogenetics" refers to the use of light (via lasers or LEDs) to identify or control specific components of biology. In neuroscience, optogenetics is used to understand how specific subclasses of brain cells contribute to larger neural circuits and to behavior. Optogenetics involves two steps. The first step is to inject an agent that causes the expression of channelrhodopsin (or other type of opsin), a light-sensitive ion channel, in neurons that have a particular genetic signature. After a few weeks, the opsin is expressed and a light fiber can be used to control ion channels in those particular cells.

Electrical microstimulation: Electrical microstimulation involves brief (<1 ms) pulses of electrical current between two neighboring electrodes. These currents activate cell bodies and projecting axons that propagate a volley of neural activity to downstream regions. This technique has been used for decades in neuroscience. We will use electrical microstimulation as a way to measure inter-regional information transfer and synchronization. For example, we can provide a pulse of stimulation to the thalamus while recording from the medial prefrontal cortex. Then, we will use optogenetics to silence or activate specific interneuron types and quantify the change in electrical potential. Stimulation will be titrated per animal to be the lowest

amount of stimulation required to elicit a response; the goal is not to elicit an overt behavioral response, but rather to probe inter-regional connectivity.

REFERENCES

Low RJ, Gu, Y., Tank, DW (2014). Cellular resolution optical access to brain regions in fissures: Imaging medial prefrontal cortex and grid cells in entorhinal cortex. PNAS, (111) 18739–18744.

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

All animals will undergo the same three phases of the experiment, in the same order (1-surgery, 2-recordings, 3-euthanasia). All experiments have a similar approach; differences are related to the location of implantations, sensory stimuli, and stimulation parameters.

Phase 1: Surgery

Surgery is performed to implant electrodes and inject viruses to measure and influence brain activity. We follow standard established protocols that increase scientific benefit and precision, while minimizing discomfort. Surgery is performed on healthy animals. Inhalant anesthesia is provided, and body temperature and respiration are continuously monitored during surgery. Animals will receive both pre- and post-operative analgesia and to minimize pain. Animals are checked frequently after surgery for signs of distress or sickness (e.g., eating and grooming habits, fur quality, etc.).

Because of viral injections, surgeries take place in a class-II biosafety cabinet. This necessitates performing two surgeries, one for viral injections inside the biosafety cabinet, and a second surgery 1-3 weeks later for implanting electrodes and/or lens. Depending on the target, we will use either replication-defective Adeno Associated viruses (AAV)/CAV2/G-deleted or concentrated replication-incompetent, self-inactivating lentivirus. Different viruses are used to target different subclasses of cells, but none of the viruses are associated with any discomfort, behavioral impairments, or bodily harm. Instead, the virus causes the production of opsins in neurons that allow us to provide microstimulation to briefly excite or inhibit those neurons.

State-of-the-art electrodes will be implanted to record the activities of several dozens of neurons and neural circuits simultaneously. Improved electrode technology allows for smaller and thinner probes (meaning less damage to the brain as the electrode is implanted). Depending on the experiment protocol, electrodes will be implanted into the cortex or subcortical structures. Surgical implantation is done via digitally controlled manipulator arms that provide very high targeting precision, based on standardized brain atlases.

After implantation, the electrodes and fibers are secured to the skull. This allows chronic recordings for up to a maximum of 10 months, or less if humane endpoints are reached, maximizing the amount of data generated per animal and minimizing the total numbers of animals utilized. Electrodes and optical fiber are secured to a headpost, which is like a cap that is secured to the skull via screws and bone cement. The headpost is 3D-printed to be strong yet light. Animals quickly adjust to the presence of the headpost and are not impeded by it during daily activities. We have gained considerable expertise at designing and implanting these devices, and are confident that it causes minimal discomfort while maximizing scientific benefit. We use special-designed cages that are taller to prevent the headpost from accidentally hitting the top part of the cage. Cages include enrichment and nesting material.

Recovery time from each surgery is typically 3-5 days.

Selecting animals for imaging or electrophysiology
The calcium imaging experiments involve implanting a lens in an opening in the skull. For this procedure to be safe, there cannot be large veins near the target site (otherwise we risk damaging the brain's blood supply, which can be fatal). We will follow the following established procedure (reported by Low et al., 2014): Each animal will be screened by skull-thinning to examine the local vasculature. Approximately 40% of animals have bridging veins that would prevent safe implantation of the lens; these animals will not be used for imaging and instead will be used only for electrophysiology studies (the electrophysiology surgery involves only very small burrholes in the skull; veins are easily avoided). This decision is made at the start of the surgery, in order to minimize the number of surgeries that are necessary.

Phase 2: Tasks and recordings
Mice will be tested in awake head-fixation during behavior tasks and resting-state (spontaneous recordings). The use of chronic non-anesthetized animals is a strong advantage because it minimizes discomfort and produces a very large amount of data from each individual. Animals can be tested up to five days per week for up to 90 minutes per day (less time if there are signs of discomfort). These are theoretical maximum possible recording durations; in practice, scheduling, equipment availability, and animals' motivation limit the actual number and duration of recordings. Brain electrical activity is recorded continuously during the testing sessions. In addition, cameras and breathing monitors can be made available for behavior and physiology to be monitored.

Each experiment will include 12 animals per group. A statistical justification for this number is provided in the next section. Some behavioral training will begin prior to surgery to familiarize the mice to the experimental setup and stimuli. Head fixation is gradually introduced through handling and rewards, following standard procedures used at many universities and neuroscience research institutes.

Reward learning task (three sensory modalities [visual, auditory, and whisker] in five groups [SOM, PVB, VIP, Pyr-deep, Pyr-super] and for two imaging methods [imaging, electrophysiology-only]). The purpose of these tasks is to investigate the local and long-range neural circuitry underlying reward learning-related interactions between the executive functioning network and sensory processing regions. Mice will undergo operant reward conditioning. Stimuli of different modalities (visual, auditory, whisker) will be paired with liquid rewards, while other stimuli will not be paired with rewards. Licking spouts will be available to indicate behavioral responses. Optogenetic silencing will take place during or after sensory stimuli or rewards are delivered.

Mice will also undergo "resting tasks," in which no specific behaviors are required. These recordings provide an important comparison condition for the task-related dynamics as well as the opportunity to make more detailed assessments of the roles of interneurons in synchronization.

Our calculations indicate that we will need approximately 40 hours of recordings per animal. This is based on the anticipated number of stimulation frequencies and repetitions (e.g., 100 frequencies X 100 trials X 10 seconds per trial ~= 30 hours), and learning conditions. This cannot be done in a single session, therefore the sessions are spread out over time. Assuming ~30 minutes of recordings per session and ~3 sessions per week (although it is possible to have longer sessions, these are realistic numbers based on scheduling and availability of shared equipment), we anticipate requiring 7 months per animal (including peri-surgery adjustment, recuperation, and training). On the other hand, there are occasional delays in the institute that are outside of our control, and it is sometimes necessary to pause data collection for days or weeks at a time. Therefore, we allow a

potential maximum of up to 10 months for data collection. We stress that these are the maximum possible limits; in practice, data collection will not take the maximum amount of time.

Description of experiment outline for a single animal
To help clarify what will happen in the experiment, we here describe an example of the procedure for a single animal (the experience for all animals is similar, with differences due to sensory modalities, genotype, and so on). At the start of a recording session, the animal will be placed into the head-fixation on a styrofoam ball inside the dome. By this point, the animal has had considerable experience being handled by the researcher and is familiar with the setup. Furthermore, the animal has learned that the experiment provides liquid rewards. This step is therefore not associated with significant discomfort. Attaching the electrode and fiber from the animal to the equipment takes a few minutes, and then the recordings begin. During the learning task, large and easily discriminable visual objects will appear on the screen (for example, a circle or a triangle). Liquid rewards will accompany one stimulus but not the other stimulus. The reward is available via a dispenser placed close to the mouse's mouth. The mouse simply has to lick to retrieve the reward (we record these licks using sensitive piezos; this provides behavioral evidence of learning). Within a few minutes, the animal learns that one stimulus predicts a reward while the other doesn't. We will test the hypothesis that this learning is accompanied by increases in synchronization between sensory areas and prefrontal/striatal areas. In some conditions, we will use the light fiber to inhibit the activity of molecularly defined interneurons, in order determine their contribution to learning and neural synchronization. Because of the simplicity of the task, the animal will learn the stimulus-reward association within a few minutes; for this reason, different recording sessions will use different stimuli (for example, circles and triangles, or squares and diamonds; complex pictures are also used when they are easily discriminable and matched for low-level visual features). The animal will need to learn new associations each time, which allows us to investigate the learning process over repeated trials.

During the passive "resting tasks" used for stimulation, the animal will remain in the setup but will not perform a task. We will apply brief pulses of electrical stimulation to induce activity, while using the light fiber to inhibit the activity of interneuron populations. This will provide detailed information regarding the contribution of interneuron types to local and inter-regional synchronization. The advantage of these recordings is that we have better control over the patterns of synchronization compared to the endogenous dynamics that appear during learning. The stimulation levels are too low to produce any observable behavioral response, and thus we do not anticipate that the animal will experience discomfort resulting from this stimulation. Animals will receive occasional liquid rewards to maintain their motivation level.

The recording session described above ends (1) after a maximum of 90 minutes, (2) depending on equipment scheduling, or (3) the animal is not motivated to continue. Signs of this latter case include struggling or lack of interest in the liquid rewards. In any of these cases, the animal is immediate removed from the setup and returned to the home cage.

Electrical and optogenetic stimulation
Stimulation is necessary to establish causality. For example, we hypothesize that the mediodorsal thalamus acts as a gate for sensory information to enter the medial prefrontal cortex. We therefore expect that silencing the thalamus will cause a reduction of medial prefrontal theta oscillations. This is a crucial aspect of the research and it is not possible to test these hypotheses using alternative methods.

Electrical microstimulation has been the standard method for transiently perturbing neural networks. Although it is temporally and spatially precise, it lacks any cell-type specificity because it activates all neurons within the current field, and therefore is not very physiologically realistic.

Optogenetic stimulation is equally temporally and spatially precise but also has very high cell-type specificity. It also allows for inhibition, which is not possible with electrical stimulation. But it is also a new technique. We need to compare it to electrical microstimulation in order to determine their differences and to facilitate comparison to the existing literature. Furthermore, combined electrical and optogenetic stimulation gives additional flexibility for investigating 3-way network interactions (e.g., using optogenetics to silence SOM interneurons in the medial prefrontal cortex while providing electrical microstimulation to the thalamus).

Phase 3: Euthanasia and post-experiment anatomical confirmation
To achieve interpretable results, it is important that all animals remain healthy and show no signs of stress or reduced well being during all experimental procedures. Indeed, only healthy animals can provide the best quality data. Animals will be checked daily for any signs of sickness. Humane endpoints of the research are reached by overt signs of sickness, loss of body weight (more than 15%) or other indications of reduced well being.

If discomfort levels appear to be unusually high (immobility, refraining from drinking or eating, pale color of eyes and skin), the animal will be euthanized and a post-mortem examination will be performed with the aim of understanding possible causes and potentially further refining our procedures.

Euthanasia will be performed with an injection of pentobarbital in isoflurane anesthetized animals. After carefully checking the level of anesthesia (absence of tail-pinch reflex), perfusion with paraformaldehyde will be completed in order to remove and section the brain for histological verification of electrodes and fiber placements, and viral expression.

Pilots
It is important to optimize our surgical and experimental techniques. This will improve the scientific quality of the data as well as minimizing discomfort for the animals. We therefore request an additional 35 (WT) animals for piloting (15 for behavioral task piloting, and 20 for surgery piloting). These will be used to test surgical procedures and ensure that the tasks are sufficiently well-designed.

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

STATISTICS FOR NEURAL DATA
For the neural data, we focus on time-frequency-based analyses of oscillations, synchronization, and spike-field coherence. The primary PI involved in this research is an expert in these kinds of data analyses, as exemplified by his 600-page textbook on neuroscience data analyses and statistics [redacted] Each animal will provide many thousands of trials of data, ensuring a large probability of high-statistical-power results. Statistical analyses vary depending on the specific analysis, but mostly involve either (1) non-parametric permutation-based testing or (2) parametric test such as ANOVAs or multiple regressions. Multiple comparisons issues are addressed using: (1) cluster- or maximum-pixel correction for time-frequency-space analyses; (2) Bonferroni correction across groups; (3) split-half replication, in which all analyses are performed on 50% of the data, and the findings are confirmed in the other 50% of the data. Statistics and multiple comparisons corrections will follow standard procedures in neuroscience (Maris and Oostenveld, 2007).

Most of our analyses will focus on changes in neural activity before vs. after brief pulses of stimulation and sensory/reward stimuli. We will compare action potentials, local field potential signals in different frequency bands, EEG activity, and inter-regional synchronization.

CALCULATION OF THE NUMBER OF ANIMALS PER GROUP

Having a sufficient sample size is crucial in neuroscience experiments (Button et al., 2013; Nieuwenhuis et al., 2011). In total, we will utilize 12 animals per group. This number is derived from power analyses of previous literature. For example, the effect sizes from one study using similar analyses (van Wingerden et al., 2014) suggests that 11 animals is sufficient for a power of beta=.8 and alpha=.05. Having 12 animals also provides some buffer in case we need to exclude data if a few animals are not able to complete the tasks, if there are technical problems, or if humane endpoints are reached.

On the other hand, we acknowledge that because of the novelty of our analyses, appropriate sample sizes are not known for all of our analyses. We will closely monitor our results, and if pilot testing reveals that 12 animals per group is more than sufficient, fewer animals may be used.

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van Wingerden M, van der Meij R, Kalenscher T, Maris E, Pennartz CM. (2014). Phase-amplitude coupling in rat orbitofrontal cortex discriminates between correct and incorrect decisions during associative learning. J Neurosci. 2014 Jan 8;34(2):493-505.

B. The animals

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

We will use six types of mice. Five types are so-called Cre-driver line mice, meaning that they are genetic lines that express Cre-recombinase in molecularly identified neuron subclasses. This allows us to target specific subclasses on neurons. In the previous sections we outlined the justification of animal numbers for each experiment. The sixth group is WT animals that are used for piloting and optimizing surgical and experimental techniques. We will use males and females.

None of the gene lines we will use are associated with physiological impairments or genetic-caused distress or discomfort.

SOMcre, PVBcre, and VIPcre: These animals allow us to target somatostatin-positive, parvalbumin-positive, and vasoactive intenstinal peptide-positive cells. As mentioned earlier, these cells are thought to make different contributions to neural computations. We will evaluate the roles of these interneuron types to network oscillations and reward learning.

PyrDeep, PyrSuper: These animals allow us to target excitatory pyramidal cells in the deep vs. superficial layers of the cortex. This is important because the different cortical layers subserve different functional roles in network synchronization and cognition.

Species	Origin	Maximum number of animals	Life stage
Mouse (SOMcre)	Own breeding colony	72	Adult
Mouse (PBVpre)	Own breeding colony	72	Adult
Mouse (VIPcre)	Own breeding colony	72	Adult
Mouse (PYRdeep)	Own breeding colony	72	Adult
Mouse (PYRsuper)	Own breeding colony	72	Adult
Mouse (WT, pilots)	Own breeding colony	35	Adult

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.

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

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement.
There are several large-scale efforts to develop accurate computational models of the brain or brain subsystems. Though promising, these models still require confirmation from empirical studies, and are not ready for use in scientific or clinical research. In our own research, we are working on a computational model of medial frontal cortex functioning that will be informed by this research. However, at present, the kinds of experiments discussed in this proposal are necessary to develop a better understanding of brain function. Thus, it is currently not possible to replace animal

models with computer simulations. Furthermore, lower animal species such as insects do not have sufficient cognitive capabilities or brain development to make them valid models for understanding cognitive function in humans. Rodents share several cognitive functions with humans, including sensory decision-making and working memory. Furthermore, the availability of Cre driver lines is a strong argument in favor of using mice, because these lines allow us to investigate the contributions of specific subclasses of neurons to neural and cognitive functions.

The primary measurement techniques used here -- invasive electrophysiology and optical imaging -- are generally not available in humans. There are some clinical situations in which subdural electrodes are placed (e.g., epilepsy or deep-brain-stimulation), but these recordings are generally made from unhealthy tissue, these patients are rare and difficult to access, and the recordings often do not include activity from individual cells. Thus, rodents are necessary and at present cannot be replaced by another species.

Reduction.
Our experiments are designed with the minimal number of animals needed to answer the research questions, while having a sufficient number of animals for high statistical power. As mentioned earlier, there are two important components of our research that work towards reduction of the number of animals that will be tested. First, we obtain high-density multisite chronic recordings from each animal. This sharply reduces the number of animals we need to test to obtain sufficient data. Second, we will make all of our collected data publicly available for other scientists to download and use. This reduces the number of animals tested by other research groups around the world, because several hypotheses can be tested in our data, rather than having to collect new data.

Refinement.
All procedures with the animals will be performed by experienced researchers/caretakers to keep the discomfort for the animals as low as possible. We believe that only healthy animals with minimal stress can provide the high-quality scientific data that we seek. Therefore, we are highly motivated to refine our procedures as best as possible. The procedures and models we use in this proposal are described in literature to give reliable data. Furthermore, we are continuously monitoring and discussing our procedures to minimize discomfort and improve the health and well-being of the animals. A few specific examples are listed below (see also section H3).
- Recent innovations in 3D printing technology now allow us to use lighter and smaller head-mounted electrode holders.
- We have discovered that mice appear more comfortable by making specific adjustments to the head-fixation device (e.g., its angle relative to the floor surface).
- We begin animal handling prior to surgery so that animals are used to human interactions prior to physical discomfort; this reduces stress when being handled after surgery.
- Animals are provided supervised "social play time," in which they can interact with their peers in a cage for 10-30 minutes. Interactions must be supervised by researchers to prevent other animals from damaging the headstages. In addition to reducing overall stress and discomfort, play-time acts as another motivator for animals to perform the tasks and remain calm during testing sessions.

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

Our procedures are guided by strong considerations for the welfare of the animals. Unhealthy or stressed animals provide bad data, and it is thus obvious from both scientific and welfare perspectives that the animals must be kept as healthy and comfortable as possible. Furthermore, we

continuously seek to improve our procedures, and this topic is often discussed in group meetings. Section H3 details sources of discomfort and how we will seek to minimize or mitigate them. A few specific examples of how we strive to minimize discomfort were provided in the previous section.

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.

Non-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.

Following is a list of components of the research that may cause discomfort, and how we will minimize discomfort.

Initial arrival. After arrival or transfer from the breeding facility, animals will be allowed to adapt to the animal facility for at least 7 days. They will be regularly weighed and checked for general health. All animals will be handled to familiarize them to the experimenter and the experimental procedures. Cage enrichment is available.

Surgical implantation of electrodes and viruses. Animals will be weighed and anesthetized with isoflurane (4-5% for induction, reduced during surgery) according to standard protocol. During surgery, animals will be placed on a heating pad and their heart rate and blood oxygenation levels will be monitored continuously. After thorough cleaning of the skin surface, lidocaine (2-4%) will be injected locally around the incision site. Surgery is performed while the animal is under isofluorane-maintained anesthesia.

NaCl will be given subcutaneously to keep the mice hydrated as needed. Carpofen is administered at least 30 minutes prior to the end of surgery, at 5 mg/kg bodyweight. Animals will be kept under observation at 30C, and moved to their cage after approximately after 1 hour where they will have free access to food and water post-surgery. 24 hours after surgery animals are given carprofen. During one week after surgery their behavior, appearance, and weight are monitored closely, once per day (or more frequently if necessary).

Post-surgical procedures. Animals will receive carprofen (s.c.) after surgery as an analgesic. We will also use buprenorphine (in saline s.c.) if the animal is visibly in pain. This will be repeated 24 hours later and again 48 hours later. Saline (at body temperature) will be administered s.c. to prevent dehydration as needed. A heating pad will be placed under the recovery cage to further facilitate post-anesthesia recovery, and will be closely monitored until fully recovered from anesthesia. Once awake, rodents will be placed in their home cage in an independently ventilated cage

(IVC) located in a DM-II room for a 2-week quarantine period. After said period, animals may be considered virus free and returned to conventional lab space. Animals will be weighed and checked for general health and recovery from surgery (wound recovery, mobility, etc.) daily for minimum of 3 consecutive days, but typically 5 days, until they regain their pre-operative weight. Animals that do not adequately recover from surgery and show reduced signs of well-being (loss of more than 15% of pre-operative body weight) will be euthanized in accordance with humane end points. Several research groups at the institute have extensive experience with these general stereotaxic techniques and have developed a highly standardized protocol for both the surgical procedures as well as pre-, peri- and post-operative care. Animal welfare logbooks will be kept. Rodents will be allowed to fully recover before commencement of the experiment procedures.

Housing. Single housing is necessary for this research because of the headpost that is attached to the head. Other mice may bite or scratch at this construction, which would cause damage and could lead to infection or death. However, single housing can cause moderate discomfort for mice, because they are social animals. All cages are kept in close proximity, meaning animals can see, hear, and smell each other. Animals are checked daily for signs of sickness or stress, and are frequently weighed (typically, daily). Cages include enrichment and nesting materials. No shelters are not included to avoid damaging the headpost.

We will provide "supervised play-time" for the animals. This means putting animals together in the same cage to engage in social interactions, for periods of 10-30 minutes. Researcher/caretaker supervision is necessary to make sure the animals are not damaging each other's implants (this could cause irritation or infection). This play-time reduces stress and, when offered after training/experiment sessions, provides a natural motivator for good behavior during the recordings.

Food and water scheduling. Water or food scheduling is necessary to keep animals motivated to learn and perform the tasks. In general, we prefer water scheduling over food restriction for three reasons: It causes less discomfort, it is easier to regulate, and it has a faster time-course (animals get thirstier faster than they get hungry). Food restriction is used when water scheduling is insufficient. However, excessive restriction is neither necessary nor desirable. Animals will be kept at approximately 90% body weight during training and during early phases of the recordings, estimated through standard growth curves (e.g., through information from Jax). If weight drops below 85%, ad libitum food and water are provided until they return to normal weight. Restriction becomes less necessary as the animals are familiarized and trained on the task.

Behavioral tasks. The tasks are not associated with any direct discomfort (no punishments, shocks, or other aversive stimuli are used). All tasks involve some reward component (typically, liquid reward delivered through spouts). Recording sessions will last no longer than 90 minutes per day, or shorter if there are signs of stress or discomfort, for up to five days per week. Head-fixation causes discomfort, particularly in the beginning. Animals are introduced to head-fixation slowly and are given rewards like sucrose water, soy milk, and sweet yogurt. Within days to a few weeks, animals learn that the head-fixation is safe, is associated with rewards, and does not last very long.

Stimulation. Based on literature and our previous experience, focused brain stimulation techniques do not cause any noxious effects. The stimulation is titrated per animal to be as minimal as possible while producing a measurable effect.

I. Other aspects compromising the welfare of the animals

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

see H3

Explain why these effects may emerge.

see H3

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

H3

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.

Humane endpoints include overt signs of sickness or clinical discomfort (for example, dehydration, 15% weight loss in less than 2 days, or a decrease of 20% compared to the weight at start of the experiment). Other risks arise from errors or infections during surgery and implantations. Needless to say, each animal is important and we do our best to avoid unnecessary risks or infections. In our experience, humane endpoints resulting from experimenter or surgical errors in <5% of cases. In such events, discussions are held to determine the source of the incident and strategies to improve our techniques.

Indicate the likely incidence.

Based on previous research and our experience, we anticipate <5%.

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).

We expect that 95% of the animals will experience severe discomfort resulting from the surgery and recover from anesthesia, and from single-housing (animals are in continuous smell/sound range of each other, which mitigates this source of discomfort to some extent). Even in the exceptional situation of an infection (<5%), pain management drugs and timely application of the humane endpoints will prevent extreme discomfort. Animals used for behavior piloting will not undergo surgery or anesthesia, and may experience mild discomfort from food or water scheduling.

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.

It is necessary to confirm the expression of the virus and placement of the electrodes in post-mortem imaging of the brain.

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

Centrale Commissie Dierproeven

5.

> 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
AVD103002017873

Bijlagen
2

Datum 17 februari 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted],

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 17 februari 2017. Het gaat om uw project "Interneurons, oscillations, and learning". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD103002017873. 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:
17 februari 2017
Aanvraagnummer:
AVD103002017873

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
17 februari 2017
Aanvraagnummer:
AVD103002017873

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 10
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:
17 februari 2017
Aanvraagnummer:
AVD103002017873

Gegevens verantwoordelijke uitvoering proces

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

Over uw aanvraag

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

Over uw project

Geplande startdatum:	17 maart 2017
Geplande einddatum:	17 maart 2022
Titel project:	Interneurons, oscillations, and learning
Titel niet-technische samenvatting:	Neuronen, oscillaties, en leerprocessen
Naam DEC:	RU DEC
Postadres DEC:	Postbus 9101, 6500 HB Nijmegen[redacted]
E-mailadres DEC:	[redacted]

Betaalgegevens

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

Checklist bijlagen

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

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Nijmegen
Datum:	17 februari 2017

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Geert Grooteplein 29
Postbus 9101, [redacted]
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
AVD103002017873

Bijlagen
2

Datum 17 februari 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 17 februari 2017
Vervaldatum: 19 maart 2017
Factuurnummer: 170873
Ordernummer: Kostenplaats en kostensoort: 040823-461220 [redacted]
projectnummer: 2016-0079 Verantwoordelijk onderzoeker: [redacted]

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD103002017873	€ 1035,00

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

Centrale Commissie Dierproeven

6.

> 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
AVD103002017873

Datum 27 februari 2017
Betreft aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 17 februari 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Interneurons, oscillations, and learning" met aanvraagnummer AVD103002017873. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

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

Niet technische samenvatting
De formulering 'kamer op de kop' zal door het algemene publiek niet in context geplaatst kunnen worden. Kunt u beschrijven hoe groot dit ongeveer is, en hoeveel last het dier er van zal hebben.
Bij de 3V's beschrijft u dat uw experimenten/ resultaten zullen zorgen dat er in de toekomst geen dierexperimenten uitgevoerd hoeven worden. Dit ligt buiten uw invloedssfeer en kan daarom niet op deze manier aan het algemeen publiek gecommuniceerd worden. Kunt u dit anders formuleren.
U beschrijft dat de dieren nooit vervelende of pijn prikkels krijgen. Dit is niet in overeenstemming met de water- en voedsel deprivatie die u beschrijft. Kunt u dit herformuleren?
U beschrijft dat de dieren matig ongerief zullen ondervinden. in de bijlage dierproeven beschrijft u dat 95% van de dieren ernstig ongerief zal ondervinden. Kunt u dit aanpassen?

Datum:
27 februari 2017
Aanvraagnummer:
AVD103002017873

Onduidelijkheden
In de bijlage Dierproeven heeft u bij F. aangekruist dat de huisvesting van de dieren niet afwijkt van bijlage III van de richtlijn. Door de individuele huisvesting is dit wel afwijkend van bijlage III. Kunt u dit aanpassen?

U heeft in de bijlage dierproeven beschreven onder punt K. dat 95% van de dieren ernstig ongerief zal ondervinden. Kunt u aangeven wat het ongerief van de overige 5% van de dieren zal zijn, en de bijlage aanpassen?

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

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

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

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

Met vriendelijke groet,

Centrale Commissie Dierproeven

Bijlagen:
- Melding bijlagen
- Niet technische samenvatting

Centrale Commissie Dierproeven

Melding bijlagen

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

1 Uw Gegevens

Naam instelling:	Stichting Katholieke Universiteit Nijmegen
Adres:	.....
Postcode en plaats:	.....
Aanvraagnummer:	AVD103002017873

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

O Projectvoorstel

O Beschrijving Dierproeven

O Niet-technische samenvatting

O Melding Machtiging

O Aanvraagformulier

O .....

O .....

O .....

Datum:
27 februari 2017
Aanvraagnummer:
AVD103002017873

3 Ondertekening

Naam: .....

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

Handtekening: .....

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

7.

Datum - 27 februari 2017
Betreft - aanvraag projectvergunning Dierproeven

Geachte Centrale Commissie Dierproeven,

Thank you for the evaluation of this proposal ("Interneurons, oscillations, and learning"; aanvraagnummer AVD103002017873). I was pleased to read the overall positive evaluation. The committee also noted a few points in the proposal that needed further clarification before a final decision could be made. I thank the committee for their time and efforts to ensure that this ethics proposal is complete. Below is a point-by-point response to the comments.

De formulering 'kamer op de kop' zal door het algemene publiek niet in context geplaatst kunnen worden. Kunt u beschrijven hoe groot dit ongeveer is, en hoeveel last het dier er van zal hebben.
Reply
The description of the recording chamber was reformulated. It is now referred to more concretely as elektroden aansluiting.

Bij de 3V's beschrijft u dat uw experimenten/ resultaten zullen zorgen dat er in de toekomst geen dierexperimenten uitgevoerd hoeven worden. Dit ligt buiten uw invloedssfeer en kan daarom niet op deze manier aan het algemeen publiek gecommuniceerd worden. Kunt u dit anders formuleren.
Reply
That is true. The meaning of the statement is that because we will make all of our data available to the scientific community, other scientists can re-use our data instead of collecting new data. But that on its own does not prevent other scientists from collecting new data. That claim was removed.

U beschrijft dat de dieren nooit vervelende of pijn prikkels krijgen. Dit is niet in overeenstemming met de water- en voedsel deprivatie die u beschrijft. Kunt u dit herformuleren?
Reply
That was indeed confusing. That statement referred to using only rewards and not shocks during the experiment, but indeed water and food restriction can be unpleasant. The statement (“De dieren krijgen nooit vervelende of pijn prikkels”) was removed.

U beschrijft dat de dieren matig ongerief zullen ondervinden. In de bijlage dierproeven beschrijft u dat 95% van de dieren ernstig ongerief zal ondervinden. Kunt u dit aanpassen?
Reply
Apologies for the inconsistency. The NTS has been adjusted to reflect that the main application lists ernstig ongerief.

In de bijlage Dierproeven heeft u bij F. aangekruist dat de huisvesting van de dieren niet afwijkt van bijlage III van de richtlijn. Door de individuele huisvesting is dit wel afwijkend van bijlage III. Kunt u dit aanpassen?
Reply

“Yes” is now selected, and we provided a justification of the individual housing, which minimizes damage to the headpost and therefore minimizes the risk of infection or other complications.

U heeft in de bijlage dierproeven beschreven onder punt K. dat 95% van de dieren ernstig ongerief zal ondervinden. Kunt u aangeven wat het ongerief van de overige 5% van de dieren zal zijn, en de bijlage aanpassen?

Reply

This is now clarified: The other animals are from the pilot-testing group, who will experience mild discomfort resulting from handling and water/food restriction (behavioral task piloting), or from anesthesia induction (surgical piloting).

8.

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 Description of procedures for surgery and tasks.

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.

METHODS TO MEASURE BRAIN ACTIVITY, AND PRIMARY OUTCOME MEASURES

Electrophysiology: In-vivo electrophysiology is a technique that allows us to monitor ongoing brain activity in real-time during awake behavior or sleep. We implant 1-4 very thin probes into the brain that have dozens to hundreds of tiny electrodes. Each electrode acts as an antenna to record the activity of individual brain cells, as well as the local field potential (the aggregated activity over hundreds of cells). Placing electrodes in different parts of the brain also allows us to measure the interactions across brain regions, which are believed to be of central importance to brain functions like perception, memory, and thinking. Primary outcome measures: action potentials from individual neurons, local field potentials

EEG: EEG is one of the primary brain imaging methods in humans. However, EEG measures only aggregated activity summed over millions of neurons. One of the themes of our research is to understand the origin and significance of EEG. Thus, skull EEG will be measured alongside the electrophysiology. From a practical and welfare perspective, EEG adds no discomfort—the setup already requires 4-10 metal skull mounts that secure the headstage; we will simply attach wires to use as electrodes. Primary outcome measure: EEG activity (oscillations in different frequency bands)

Calcium imaging: Calcium imaging is a technique in which a lens is chronically implanted in or on the brain. During recording sessions, the lens is connected to a camera. The camera images fluorescence that is given off by activation of calcium channels, one of the key currents in active neurons. AAV viruses in combination with Cre driver lines provide these fluorescence proteins only to neurons that have a specific molecular marker, such as somatostatin for SOM interneurons. This technique therefore allows us to measure the activity of several dozens of individual cells simultaneously and reliably over time. The use of calcium imaging lenses in the medial prefrontal cortex is well established (Low et al., 2014) and used by many neuroscience research groups in the world, including the Netherlands. Primary outcome measure: activity of cells identified in images

METHODS TO CAUSALLY PROBE BRAIN ACTIVITY

Optogenetics: "Optogenetics" refers to the use of light (via lasers or LEDs) to identify or control specific components of biology. In neuroscience, optogenetics is used to understand how specific subclasses of brain cells contribute to larger neural circuits and to behavior. Optogenetics involves two steps. The first step is to inject an agent that causes the expression of channelrhodopsin (or other type of opsin), a light-sensitive ion channel, in neurons that have a particular genetic signature. After a few weeks, the opsin is expressed and a light fiber can be used to control ion channels in those particular cells.

Electrical microstimulation: Electrical microstimulation involves brief (<1 ms) pulses of electrical current between two neighboring electrodes. These currents activate cell bodies and projecting axons that propagate a volley of neural activity to downstream regions. This technique has been used for decades in neuroscience. We will use electrical microstimulation as a way to measure inter-regional information transfer and synchronization. For example, we can provide a pulse of stimulation to the thalamus while recording from the medial prefrontal cortex. Then, we will use optogenetics to silence or activate specific interneuron types and quantify the change in electrical potential. Stimulation will be titrated per animal to be the lowest

amount of stimulation required to elicit a response; the goal is not to elicit an overt behavioral response, but rather to probe inter-regional connectivity.

REFERENCES

Low RJ, Gu, Y., Tank, DW (2014). Cellular resolution optical access to brain regions in fissures: Imaging medial prefrontal cortex and grid cells in entorhinal cortex. PNAS, (111) 18739–18744.

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

All animals will undergo the same three phases of the experiment, in the same order (1-surgery, 2-recordings, 3-euthanasia). All experiments have a similar approach; differences are related to the location of implantations, sensory stimuli, and stimulation parameters.

Phase 1: Surgery

Surgery is performed to implant electrodes and inject viruses to measure and influence brain activity. We follow standard established protocols that increase scientific benefit and precision, while minimizing discomfort. Surgery is performed on healthy animals. Inhalant anesthesia is provided, and body temperature and respiration are continuously monitored during surgery. Animals will receive both pre- and post-operative analgesia and to minimize pain. Animals are checked frequently after surgery for signs of distress or sickness (e.g., eating and grooming habits, fur quality, etc.).

Because of viral injections, surgeries take place in a class-II biosafety cabinet. This necessitates performing two surgeries, one for viral injections inside the biosafety cabinet, and a second surgery 1-3 weeks later for implanting electrodes and/or lens. Depending on the target, we will use either replication-defective Adeno Associated viruses (AAV)/CAV2/G-deleted or concentrated replication-incompetent, self-inactivating lentivirus. Different viruses are used to target different subclasses of cells, but none of the viruses are associated with any discomfort, behavioral impairments, or bodily harm. Instead, the virus causes the production of opsins in neurons that allow us to provide microstimulation to briefly excite or inhibit those neurons.

State-of-the-art electrodes will be implanted to record the activities of several dozens of neurons and neural circuits simultaneously. Improved electrode technology allows for smaller and thinner probes (meaning less damage to the brain as the electrode is implanted). Depending on the experiment protocol, electrodes will be implanted into the cortex or subcortical structures. Surgical implantation is done via digitally controlled manipulator arms that provide very high targeting precision, based on standardized brain atlases.

After implantation, the electrodes and fibers are secured to the skull. This allows chronic recordings for up to a maximum of 10 months, or less if humane endpoints are reached, maximizing the amount of data generated per animal and minimizing the total numbers of animals utilized. Electrodes and optical fiber are secured to a headpost, which is like a cap that is secured to the skull via screws and bone cement. The headpost is 3D-printed to be strong yet light. Animals quickly adjust to the presence of the headpost and are not impeded by it during daily activities. We have gained considerable expertise at designing and implanting these devices, and are confident that it causes minimal discomfort while maximizing scientific benefit. We use special-designed cages that are taller to prevent the headpost from accidentally hitting the top part of the cage. Cages include enrichment and nesting material.

Recovery time from each surgery is typically 3-5 days.

Selecting animals for imaging or electrophysiology
The calcium imaging experiments involve implanting a lens in an opening in the skull. For this procedure to be safe, there cannot be large veins near the target site (otherwise we risk damaging the brain's blood supply, which can be fatal). We will follow the following established procedure (reported by Low et al., 2014): Each animal will be screened by skull-thinning to examine the local vasculature. Approximately 40% of animals have bridging veins that would prevent safe implantation of the lens; these animals will not be used for imaging and instead will be used only for electrophysiology studies (the electrophysiology surgery involves only very small burrholes in the skull; veins are easily avoided). This decision is made at the start of the surgery, in order to minimize the number of surgeries that are necessary.

Phase 2: Tasks and recordings
Mice will be tested in awake head-fixation during behavior tasks and resting-state (spontaneous recordings). The use of chronic non-anesthetized animals is a strong advantage because it minimizes discomfort and produces a very large amount of data from each individual. Animals can be tested up to five days per week for up to 90 minutes per day (less time if there are signs of discomfort). These are theoretical maximum possible recording durations; in practice, scheduling, equipment availability, and animals' motivation limit the actual number and duration of recordings. Brain electrical activity is recorded continuously during the testing sessions. In addition, cameras and breathing monitors can be made available for behavior and physiology to be monitored.

Each experiment will include 12 animals per group. A statistical justification for this number is provided in the next section. Some behavioral training will begin prior to surgery to familiarize the mice to the experimental setup and stimuli. Head fixation is gradually introduced through handling and rewards, following standard procedures used at many universities and neuroscience research institutes.

Reward learning task (three sensory modalities [visual, auditory, and whisker] in five groups [SOM, PVB, VIP, Pyr-deep, Pyr-super] and for two imaging methods [imaging, electrophysiology-only]). The purpose of these tasks is to investigate the local and long-range neural circuitry underlying reward learning-related interactions between the executive functioning network and sensory processing regions. Mice will undergo operant reward conditioning. Stimuli of different modalities (visual, auditory, whisker) will be paired with liquid rewards, while other stimuli will not be paired with rewards. Licking spouts will be available to indicate behavioral responses. Optogenetic silencing will take place during or after sensory stimuli or rewards are delivered.

Mice will also undergo "resting tasks," in which no specific behaviors are required. These recordings provide an important comparison condition for the task-related dynamics as well as the opportunity to make more detailed assessments of the roles of interneurons in synchronization.

Our calculations indicate that we will need approximately 40 hours of recordings per animal. This is based on the anticipated number of stimulation frequencies and repetitions (e.g., 100 frequencies X 100 trials X 10 seconds per trial ~= 30 hours), and learning conditions. This cannot be done in a single session, therefore the sessions are spread out over time. Assuming ~30 minutes of recordings per session and ~3 sessions per week (although it is possible to have longer sessions, these are realistic numbers based on scheduling and availability of shared equipment), we anticipate requiring 7 months per animal (including peri-surgery adjustment, recuperation, and training). On the other hand, there are occasional delays in the institute that are outside of our control, and it is sometimes necessary to pause data collection for days or weeks at a time. Therefore, we allow a

potential maximum of up to 10 months for data collection. We stress that these are the maximum possible limits; in practice, data collection will not take the maximum amount of time.

Description of experiment outline for a single animal
To help clarify what will happen in the experiment, we here describe an example of the procedure for a single animal (the experience for all animals is similar, with differences due to sensory modalities, genotype, and so on). At the start of a recording session, the animal will be placed into the head-fixation on a styrofoam ball inside the dome. By this point, the animal has had considerable experience being handled by the researcher and is familiar with the setup. Furthermore, the animal has learned that the experiment provides liquid rewards. This step is therefore not associated with significant discomfort. Attaching the electrode and fiber from the animal to the equipment takes a few minutes, and then the recordings begin. During the learning task, large and easily discriminable visual objects will appear on the screen (for example, a circle or a triangle). Liquid rewards will accompany one stimulus but not the other stimulus. The reward is available via a dispenser placed close to the mouse's mouth. The mouse simply has to lick to retrieve the reward (we record these licks using sensitive piezos; this provides behavioral evidence of learning). Within a few minutes, the animal learns that one stimulus predicts a reward while the other doesn't. We will test the hypothesis that this learning is accompanied by increases in synchronization between sensory areas and prefrontal/striatal areas. In some conditions, we will use the light fiber to inhibit the activity of molecularly defined interneurons, in order determine their contribution to learning and neural synchronization. Because of the simplicity of the task, the animal will learn the stimulus-reward association within a few minutes; for this reason, different recording sessions will use different stimuli (for example, circles and triangles, or squares and diamonds; complex pictures are also used when they are easily discriminable and matched for low-level visual features). The animal will need to learn new associations each time, which allows us to investigate the learning process over repeated trials.

During the passive "resting tasks" used for stimulation, the animal will remain in the setup but will not perform a task. We will apply brief pulses of electrical stimulation to induce activity, while using the light fiber to inhibit the activity of interneuron populations. This will provide detailed information regarding the contribution of interneuron types to local and inter-regional synchronization. The advantage of these recordings is that we have better control over the patterns of synchronization compared to the endogenous dynamics that appear during learning. The stimulation levels are too low to produce any observable behavioral response, and thus we do not anticipate that the animal will experience discomfort resulting from this stimulation. Animals will receive occasional liquid rewards to maintain their motivation level.

The recording session described above ends (1) after a maximum of 90 minutes, (2) depending on equipment scheduling, or (3) the animal is not motivated to continue. Signs of this latter case include struggling or lack of interest in the liquid rewards. In any of these cases, the animal is immediate removed from the setup and returned to the home cage.

Electrical and optogenetic stimulation
Stimulation is necessary to establish causality. For example, we hypothesize that the mediodorsal thalamus acts as a gate for sensory information to enter the medial prefrontal cortex. We therefore expect that silencing the thalamus will cause a reduction of medial prefrontal theta oscillations. This is a crucial aspect of the research and it is not possible to test these hypotheses using alternative methods.

Electrical microstimulation has been the standard method for transiently perturbing neural networks. Although it is temporally and spatially precise, it lacks any cell-type specificity because it activates all neurons within the current field, and therefore is not very physiologically realistic.

Optogenetic stimulation is equally temporally and spatially precise but also has very high cell-type specificity. It also allows for inhibition, which is not possible with electrical stimulation. But it is also a new technique. We need to compare it to electrical microstimulation in order to determine their differences and to facilitate comparison to the existing literature. Furthermore, combined electrical and optogenetic stimulation gives additional flexibility for investigating 3-way network interactions (e.g., using optogenetics to silence SOM interneurons in the medial prefrontal cortex while providing electrical microstimulation to the thalamus).

Phase 3: Euthanasia and post-experiment anatomical confirmation
To achieve interpretable results, it is important that all animals remain healthy and show no signs of stress or reduced well being during all experimental procedures. Indeed, only healthy animals can provide the best quality data. Animals will be checked daily for any signs of sickness. Humane endpoints of the research are reached by overt signs of sickness, loss of body weight (more than 15%) or other indications of reduced well being.

If discomfort levels appear to be unusually high (immobility, refraining from drinking or eating, pale color of eyes and skin), the animal will be euthanized and a post-mortem examination will be performed with the aim of understanding possible causes and potentially further refining our procedures.

Euthanasia will be performed with an injection of pentobarbital in isoflurane anesthetized animals. After carefully checking the level of anesthesia (absence of tail-pinch reflex), perfusion with paraformaldehyde will be completed in order to remove and section the brain for histological verification of electrodes and fiber placements, and viral expression.

Pilots
It is important to optimize our surgical and experimental techniques. This will improve the scientific quality of the data as well as minimizing discomfort for the animals. We therefore request an additional 35 (WT) animals for piloting (15 for behavioral task piloting, and 20 for surgery piloting). These will be used to test surgical procedures and ensure that the tasks are sufficiently well-designed.

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

STATISTICS FOR NEURAL DATA
For the neural data, we focus on time-frequency-based analyses of oscillations, synchronization, and spike-field coherence. The primary PI involved in this research is an expert in these kinds of data analyses, as exemplified by his 600-page textbook on neuroscience data analyses and statistics ([redacted]). Each animal will provide many thousands of trials of data, ensuring a large probability of high-statistical-power results. Statistical analyses vary depending on the specific analysis, but mostly involve either (1) non-parametric permutation-based testing or (2) parametric test such as ANOVAs or multiple regressions. Multiple comparisons issues are addressed using: (1) cluster- or maximum-pixel correction for time-frequency-space analyses; (2) Bonferroni correction across groups; (3) split-half replication, in which all analyses are performed on 50% of the data, and the findings are confirmed in the other 50% of the data. Statistics and multiple comparisons corrections will follow standard procedures in neuroscience (Maris and Oostenveld, 2007).

Most of our analyses will focus on changes in neural activity before vs. after brief pulses of stimulation and sensory/reward stimuli. We will compare action potentials, local field potential signals in different frequency bands, EEG activity, and inter-regional synchronization.

CALCULATION OF THE NUMBER OF ANIMALS PER GROUP

Having a sufficient sample size is crucial in neuroscience experiments (Button et al., 2013; Nieuwenhuis et al., 2011). In total, we will utilize 12 animals per group. This number is derived from power analyses of previous literature. For example, the effect sizes from one study using similar analyses (van Wingerden et al., 2014) suggests that 11 animals is sufficient for a power of beta=.8 and alpha=.05. Having 12 animals also provides some buffer in case we need to exclude data if a few animals are not able to complete the tasks, if there are technical problems, or if humane endpoints are reached.

On the other hand, we acknowledge that because of the novelty of our analyses, appropriate sample sizes are not known for all of our analyses. We will closely monitor our results, and if pilot testing reveals that 12 animals per group is more than sufficient, fewer animals may be used.

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Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, Munafò MR. (2013). Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci. 2013 May;14(5):365-76.
Maris E., Oostenveld, R. (2007). Nonparametric statistical testing of EEG- and MEG-data. J Neuroscience Methods. (164) 177-190.
Nieuwenhuis S, Forstmann BU, Wagenmakers EJ. (2011). Erroneous analyses of interactions in neuroscience: a problem of significance. Nat Neurosci. 2011 Aug 26;14(9):1105-7.
van Wingerden M, van der Meij R, Kalenscher T, Maris E, Pennartz CM. (2014). Phase-amplitude coupling in rat orbitofrontal cortex discriminates between correct and incorrect decisions during associative learning. J Neurosci. 2014 Jan 8;34(2):493-505.

B. The animals

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

We will use six types of mice. Five types are so-called Cre-driver line mice, meaning that they are genetic lines that express Cre-recombinase in molecularly identified neuron subclasses. This allows us to target specific subclasses on neurons. In the previous sections we outlined the justification of animal numbers for each experiment. The sixth group is WT animals that are used for piloting and optimizing surgical and experimental techniques. We will use males and females.

None of the gene lines we will use are associated with physiological impairments or genetic-caused distress or discomfort.

SOMcre, PVBcre, and VIPcre: These animals allow us to target somatostatin-positive, parvalbumin-positive, and vasoactive intenstinal peptide-positive cells. As mentioned earlier, these cells are thought to make different contributions to neural computations. We will evaluate the roles of these interneuron types to network oscillations and reward learning.

PyrDeep, PyrSuper: These animals allow us to target excitatory pyramidal cells in the deep vs. superficial layers of the cortex. This is important because the different cortical layers subserve different functional roles in network synchronization and cognition.

Species	Origin	Maximum number of animals	Life stage
Mouse (SOMcre)	Own breeding colony	72	Adult
Mouse (PBVpre)	Own breeding colony	72	Adult
Mouse (VIPcre)	Own breeding colony	72	Adult
Mouse (PYRdeep)	Own breeding colony	72	Adult
Mouse (PYRsuper)	Own breeding colony	72	Adult
Mouse (WT, pilots)	Own breeding colony	35	Adult

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.

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

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement.
There are several large-scale efforts to develop accurate computational models of the brain or brain subsystems. Though promising, these models still require confirmation from empirical studies, and are not ready for use in scientific or clinical research. In our own research, we are working on a computational model of medial frontal cortex functioning that will be informed by this research. However, at present, the kinds of experiments discussed in this proposal are necessary to develop a better understanding of brain function. Thus, it is currently not possible to replace animal

models with computer simulations. Furthermore, lower animal species such as insects do not have sufficient cognitive capabilities or brain development to make them valid models for understanding cognitive function in humans. Rodents share several cognitive functions with humans, including sensory decision-making and working memory. Furthermore, the availability of Cre driver lines is a strong argument in favor of using mice, because these lines allow us to investigate the contributions of specific subclasses of neurons to neural and cognitive functions.

The primary measurement techniques used here -- invasive electrophysiology and optical imaging -- are generally not available in humans. There are some clinical situations in which subdural electrodes are placed (e.g., epilepsy or deep-brain-stimulation), but these recordings are generally made from unhealthy tissue, these patients are rare and difficult to access, and the recordings often do not include activity from individual cells. Thus, rodents are necessary and at present cannot be replaced by another species.

Reduction.
Our experiments are designed with the minimal number of animals needed to answer the research questions, while having a sufficient number of animals for high statistical power. As mentioned earlier, there are two important components of our research that work towards reduction of the number of animals that will be tested. First, we obtain high-density multisite chronic recordings from each animal. This sharply reduces the number of animals we need to test to obtain sufficient data. Second, we will make all of our collected data publicly available for other scientists to download and use. This reduces the number of animals tested by other research groups around the world, because several hypotheses can be tested in our data, rather than having to collect new data.

Refinement.
All procedures with the animals will be performed by experienced researchers/caretakers to keep the discomfort for the animals as low as possible. We believe that only healthy animals with minimal stress can provide the high-quality scientific data that we seek. Therefore, we are highly motivated to refine our procedures as best as possible. The procedures and models we use in this proposal are described in literature to give reliable data. Furthermore, we are continuously monitoring and discussing our procedures to minimize discomfort and improve the health and well-being of the animals. A few specific examples are listed below (see also section H3).
- Recent innovations in 3D printing technology now allow us to use lighter and smaller head-mounted electrode holders.
- We have discovered that mice appear more comfortable by making specific adjustments to the head-fixation device (e.g., its angle relative to the floor surface).
- We begin animal handling prior to surgery so that animals are used to human interactions prior to physical discomfort; this reduces stress when being handled after surgery.
- Animals are provided supervised "social play time," in which they can interact with their peers in a cage for 10-30 minutes. Interactions must be supervised by researchers to prevent other animals from damaging the headstages. In addition to reducing overall stress and discomfort, play-time acts as another motivator for animals to perform the tasks and remain calm during testing sessions.

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

Our procedures are guided by strong considerations for the welfare of the animals. Unhealthy or stressed animals provide bad data, and it is thus obvious from both scientific and welfare perspectives that the animals must be kept as healthy and comfortable as possible. Furthermore, we

continuously seek to improve our procedures, and this topic is often discussed in group meetings. Section H3 details sources of discomfort and how we will seek to minimize or mitigate them. A few specific examples of how we strive to minimize discomfort were provided in the previous section.

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.

Non-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.

Single housing is necessary because other mice may bite or scratch at the headpost construction, which could cause infection or other complications. Cages are kept in close proximity to allow animals can see, hear, and smell each other. Animals are checked daily for signs of sickness or stress, and are frequently weighed (typically, daily). Cages include enrichment and nesting materials. Shelters are not included to avoid damaging the headpost.

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.

Following is a list of components of the research that may cause discomfort, and how we will minimize discomfort.

Initial arrival. After arrival or transfer from the breeding facility, animals will be allowed to adapt to the animal facility for at least 7 days. They will be regularly weighed and checked for general health. All animals will be handled to familiarize them to the experimenter and the experimental procedures. Cage enrichment is available.

Surgical implantation of electrodes and viruses. Animals will be weighed and anesthetized with isoflurane (4-5% for induction, reduced during surgery) according to standard protocol. During surgery, animals will be placed on a heating pad and their heart rate and blood oxygenation levels will be monitored continuously. After thorough cleaning of the skin surface, lidocaine (2-4%) will be injected locally around the incision site. Surgery is performed while the animal is under isofluorane-maintained anesthesia.

NaCl will be given subcutaneously to keep the mice hydrated as needed. Carpofen is administered at least 30 minutes prior to the end of surgery, at 5 mg/kg bodyweight. Animals will be kept under observation at 30C, and moved to their cage after approximately after 1 hour where they will have free access to food and water post-surgery. 24 hours after surgery animals are given carprofen. During one week after surgery their behavior, appearance, and weight are monitored closely, once per day (or more frequently if necessary).

Post-surgical procedures. Animals will receive carprofen (s.c.) after surgery as an analgesic. We will also use buprenorphine (in saline s.c.) if the animal is visibly in pain. This will be repeated 24 hours later and again 48 hours later. Saline (at body temperature) will be administered s.c. to prevent dehydration as needed. A heating pad will be placed under the recovery cage to further facilitate post-anesthesia recovery, and will be closely monitored until fully recovered from anesthesia. Once awake, rodents will be placed in their home cage in an independently ventilated cage (IVC) located in a DM-II room for a 2-week quarantine period. After said period, animals may be considered virus free and returned to conventional lab space. Animals will be weighed and checked for general health and recovery from surgery (wound recovery, mobility, etc.) daily for minimum of 3 consecutive days, but typically 5 days, until they regain their pre-operative weight. Animals that do not adequately recover from surgery and show reduced signs of well-being (loss of more than 15% of pre-operative body weight) will be euthanized in accordance with humane end points. Several research groups at the institute have extensive experience with these general stereotaxic techniques and have developed a highly standardized protocol for both the surgical procedures as well as pre-, peri- and post-operative care. Animal welfare logbooks will be kept. Rodents will be allowed to fully recover before commencement of the experiment procedures.

Housing. Single housing is necessary for this research because of the headpost that is attached to the head. Other mice may bite or scratch at this construction, which would cause damage and could lead to infection or death. However, single housing can cause moderate discomfort for mice, because they are social animals. All cages are kept in close proximity, meaning animals can see, hear, and smell each other. Animals are checked daily for signs of sickness or stress, and are frequently weighed (typically, daily). Cages include enrichment and nesting materials. No shelters are included to avoid damaging the headpost.

We will provide "supervised play-time" for the animals. This means putting animals together in the same cage to engage in social interactions, for periods of 10-30 minutes. Researcher/caretaker supervision is necessary to make sure the animals are not damaging each other's implants (this could cause irritation or infection). This play-time reduces stress and, when offered after training/experiment sessions, provides a natural motivator for good behavior during the recordings.

Food and water scheduling. Water or food scheduling is necessary to keep animals motivated to learn and perform the tasks. In general, we prefer water scheduling over food restriction for three reasons: It causes less discomfort, it is easier to regulate, and it has a faster time-course (animals get thirstier faster than they get hungry). Food restriction is used when water scheduling is insufficient. However, excessive restriction is neither necessary nor desirable. Animals will be kept at approximately 90% body weight during training and during early phases of the recordings, estimated through standard growth curves (e.g., through information from Jax). If weight drops below 85%, ad libitum food and water are provided until they return to normal weight. Restriction becomes less necessary as the animals are familiarized and trained on the task.

Behavioral tasks. The tasks are not associated with any direct discomfort (no punishments, shocks, or other aversive stimuli are used). All tasks involve some reward component (typically, liquid reward delivered through spouts). Recording sessions will last no longer than 90 minutes per day, or shorter if there are signs of stress or discomfort, for up to five days per week. Head-fixation causes discomfort, particularly in the beginning. Animals are introduced to head-fixation slowly and are given rewards like sucrose water, soy milk, and sweet yogurt. Within days to a few weeks, animals learn that the head-fixation is safe, is associated with rewards, and does not last very long.

Stimulation. Based on literature and our previous experience, focused brain stimulation techniques do not cause any noxious effects. The stimulation is titrated per animal to be as minimal as possible while producing a measurable effect.

I. Other aspects compromising the welfare of the animals

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

see H3

Explain why these effects may emerge.

see H3

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

H3

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.

Humane endpoints include overt signs of sickness or clinical discomfort (for example, dehydration, 15% weight loss in less than 2 days, or a decrease of 20% compared to the weight at start of the experiment). Other risks arise from errors or infections during surgery and implantations. Needless to say, each animal is important and we do our best to avoid unnecessary risks or infections. In our experience, humane endpoints resulting from experimenter or surgical errors in <5% of cases. In such events, discussions are held to determine the source of the incident and strategies to improve our techniques.

Indicate the likely incidence.

Based on previous research and our experience, we anticipate <5%.

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).

We expect that the animals used for the research will experience severe discomfort resulting from the surgery and recover from anesthesia, and from single-housing (animals are in continuous smell/sound range of each other, which mitigates this source of discomfort to some extent). We expect that up to 35 animals used for piloting will experience mild discomfort, resulting from handling and water/food restriction for animals used in behavioral piloting, or anesthesia induction for the animals used in the surgical pilots. Even in the exceptional situation of an infection from surgery (we anticipate this to be <5% of cases), pain management drugs and timely application of the humane endpoints will prevent extreme discomfort.

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.

It is necessary to confirm the expression of the virus and placement of the electrodes in post-mortem imaging of the brain.

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

10.

DEC-advies

A. Algemene gegevens over de procedure

1. Aanvraagnummer: 2016-0079
2. Titel van het project: Interneurons, oscillations, and learning
3. Titel van de NTS: Neuronen, oscillaties en leerprocessen

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: 23-11-2016
   ☐ aanvraag compleet
   ☒ in vergadering besproken: 06-12-2016 en 10-01-2017
   ☐ anderszins behandeld
   ☒ termijnonderbreking(en) van 12-12-2016 tot 20-12-2016 en van 17-01-2017 tot 06-02-2017
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☒ aanpassing aanvraag: 20-12-2016 en 06-02-2017
   ☒ advies aan CCD: 17-02-2017

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:
   - Datum: 10-01-2017
   - Plaats: Nijmegen
   - Aantal aanwezige DEC-leden: 8
   - Aanwezige (namens) aanvrager: [redacted]
   - Gestelde vragen: De commissie heeft de onderzoekers verzocht de achtergrond en de doelen van deze aanvraag mondeling toe te lichten, omdat zij de indruk heeft dat het de afhandeling van deze complexe aanvraag zou kunnen vereenvoudigen. De commissieleden stellen enkele vragen ter verduidelijking.
   - Verstrekte antwoorden: [redacted] geeft de gevraagde toelichting en beantwoordt de vragen.
   - Het horen van de aanvrager heeft geleid tot nieuwe vragen en opmerkingen voor de aanvrager. Deze zijn hieronder weergegeven (d.d. 17-01-2017).

9. Correspondentie met de aanvrager
   - Datum vragen: 12-12-2016
   - Datum antwoorden: 20-12-2016

   - Gestelde vragen en antwoorden:
     Project Proposal:

-3.1: Een belangrijk doel van deze projectaanvraag is te onderzoeken hoe het executieve neurale circuit is betrokken bij leerprocessen. Deze leerprocessen zijn nog onvoldoende ingeleid in de context van dit onderzoek.
Antwoord: Apologies that this was not clearly enough described. During stimulus-reward learning, neural synchronization is increased between the medial prefrontal cortex, striatum, and sensory processing regions. Our research will elucidate the roles of different neuron subclasses in changes in neural synchronization that occur during learning. Additional text has been added in sections 3.1 and 3.2.

-3.1 Klopt de bewering dat pyramidale cellen de meerderheid van de hersencellen vormen?
Antwoord: Yes, pyramidal cells comprise approximately 75% of the cells in the brain. Although interneurons are in the minority, they are extremely important for regulating the activation and timing of pyramidal cells. However, very little is known about the precise contributions of interneuron subtypes in neural synchronization and inter-regional information transfer, which is one of the reasons why the proposed research is important. This is now mentioned in section 3.1.

-3.2: De vraagstelling is onvoldoende scherp gesteld. Wat verwachten de onderzoekers te zien, in welke gebieden willen ze precies de elektrodes implanteren, wanneer is het doel bereikt? Gaan de onderzoekers het verloop van het leerproces in beeld brengen? Kunnen de onderzoekers de drie doelstellingen nog wat extra uitwerken en hun hoofddoelstelling ook hier vermelden?
Antwoord: This is now more concretely described. Perhaps part of the confusion here is that several of these questions are discussed in other parts of the proposal; I was not aware that section 3.2 should be so detailed.
I also decided to merge the first two goals because they were very similar to each other (the first was focused on local activity while the second was focused on long-range interactions, but these are similar enough to combine into one goal).

-3.4.1: Voor een muis is 16 maanden in experiment erg lang. Bovendien is de commissie van mening dat de combinatie van alle handelingen gedurende een dergelijke lange periode tot ernstig ongerief voor de dieren leidt. Waarom willen onderzoekers 5 dagen per week 16 maanden lang aan één muis meten? Is er geen moment te definiëren waarop voldoende metingen aan één muis zijn verricht?
Antwoord: Indeed, 16 months can be a long time for a mouse. We were concerned about needlessly stopping the experiment too soon, e.g., if the animal appears healthy and if there are delays for other reasons (e.g., technical difficulties or construction in the building that is out of our hands). But we understand the concern. The maximal duration is now changed to 10 months. We believe that acquiring a lot of data from individual animals helps reduce the total number of animals that need to be recorded. We would also like to point out that the animals quickly get used to the implant, that it does not interfere with their normal behavior (eating, sleeping, nesting, etc.) and that the experiment is associated with rewards.

The measurements of 90 minutes per day, 5 days per week is also a maximum. In practice there are typically fewer measurements due to scheduling, equipment availability, possible delays due to technical difficulties or building renovations, or when the animal is unmotivated. This maximum will be reached only if the equipment is available and if the animal is sufficiently motivated and healthy. This is now detailed in section 3.4.2.

Verwachten de onderzoekers dat een zo lange periode haalbaar is in de praktijk? Bovendien gaan de onderzoekers voorbij aan het feit dat de muizen in het beschreven tijdsbestek

verouderen en dus veranderen. Is het niet verstandiger deze langlopende experimenten te vervangen door korter durende experimenten met meer dieren?
Antwoord: Yes, mice age within this time-frame. We initially made our calculation based on avoiding a situation in which the experiment is delayed and the animal is healthy, and considering the balance between collecting more data in fewer animals vs. collecting less data in more animals. As written above, the maximum duration is now reduced to 10 months.

Description of Animal Procedures:
-K: De beschreven handelingen veroorzaken tezamen ernstig ongerief voor de dieren. Graag een maximum aantal maanden (zie vragen boven) aangeven voor de looptijd van het experiment.
Antwoord: The maximum number of months has been reduced to 10 months. Animals are regularly inspected for health and well-being, and the experiment will end if humane endpoints are reached. The DAP has been updated according to the modifications described above for the background sections.

- De antwoorden hebben geleid tot aanpassing van de aanvraag

- Datum vragen: 17-01-2017
- Datum antwoorden: 06-02-2017

-Deze basaal wetenschappelijke projectaanvraag richt zich op het vergaren van kennis over het optreden van oscillaties in het executieve netwerk en de bijdrage van verschillende typen interneuronen daaraan. Deze kennis is van belang om verstoringen in het executief functioneren te kunnen begrijpen en nieuwe behandelingen te ontwikkelen. Instrumenteel leren is een voorbeeld van executief functioneren. Tijdens de presentatie heeft de onderzoeker uitgelegd waarom hij juist dit voorbeeld heeft gekozen, maar die informatie ontbreekt nog in de aanvraag. Zijn de onderzoekers vooral geïnteresseerd in de oscillaties, of gaat het hen daadwerkelijk om de veranderingen hierin tijdens het leerproces? Wordt het ongestoorde leerproces ook bestudeerd? De commissie verzoekt de onderzoekers een reëel beeld te presenteren van het doel van het onderzoek: gaat het alleen over oscillaties, of is het leerproces de hoofdcomponent? (onderdeel 3.1).
Antwoord: These two aspects (learning or oscillations) are not mutually incompatible. Neural oscillations are thought to be a core mechanism by which information is processed in the brain, and information processing is fundamental to learning. On the other hand, it is important to link our fundamental findings to cognitive processes, hence the need for a behavioral task. There are many tasks we could have used that would allow us to study neural oscillations. We selected learning because it allows us to link our findings to findings in humans and to modern neuroscientific theories concerning the roles of oscillations in sensory-reward learning. The roles of different interneuron types on neural oscillations and synchronization is important for understanding healthy and abnormal human brain function, and for understanding how medications (including anti-psychotics and Parkinson's medications) alter brain activity, but it is impossible to do this kind of research in humans. Therefore, we believe this is important research that justifies the use of non-human animals.

-In aansluiting op het eerste punt wil de commissie het volgende opmerken. De onderzoekers schrijven dat zij de relatie tussen oscillaties en instrumenteel leren willen onderzoeken. In de aanvraag wordt goed uitgelegd hoe de onderzoekers de rol van interneuronen bij het optreden van oscillaties in het executief netwerk zullen onderzoeken.

De commissie mist echter een beschrijving van de stapsgewijze aanpak (strategie) waarmee de veranderingen in oscillaties tijdens het proces van instrumenteel leren worden onderzocht. Zo blijft onderbelicht welke leertaak wordt gebruikt, welke hypothesen zullen worden onderzocht en welke strategie daarvoor wordt gebruikt. Op welke manier zal de onderzoeker het leerproces en de daarbij optredende oscillaties onderzoeken? Welke stappen zijn hiervoor nodig? Een beschrijving van de experimentele handelingen is geen afdoende beschrijving van de wetenschappelijke strategie. (onderdeel 3.4)
Antwoord: Apologies that this was not clearly enough described. There is now more detail about the procedures for testing learning.

-Tijdens de presentatie heeft de onderzoeker met een berekening toegelicht waarom hij één muis gedurende zeven maanden meerdere keren wil testen. De looptijd van het experiment (nu 10 maanden) is hierop gebaseerd. Deze berekening is essentieel om de looptijd van het experiment te begrijpen, maar ontbreekt nog in de projectaanvraag. (DAP, onderdeel A)
Antwoord: The protocol is now clarified to indicate that we anticipate approximately 40 hours of recordings spread out over small recording sessions.

-De onderzoekers willen op basis van eerdere resultaten 12 dieren per groep inzetten. Uit het gevraagde aantal dieren leidt de commissie af dat de onderzoekers 6 groepen zullen testen. Een beschrijving van hetgeen in deze 6 groepen dieren getest zal worden ontbreekt nog. (DAP, onderdeel B)
Antwoord: The sixth group is wildtype animals used for piloting. I apologize that this was not clearly stated in DAP-B. The text is now adjusted. Or was the confusion about 72 animals per group divided by 12 animals per task = 6 tasks? This is because for each Cre line there are six different experiments corresponding to three sensory modalities and two brain imaging methods (3x2x12 = 72 per group). This is described in DAP-A2.

-De commissie is van mening dat het cumulatief ongerief voor de dieren door de combinatie van alle handelingen (inclusief hersenmanipulaties aan een dier bij bewustzijn) en de lange looptijd van het experiment (en daarmee van de individuele huisvesting) kan oplopen tot ernstig ongerief. (DAP, onderdeel K)
Antwoord: "Moderate" has been changed to "severe" in section DAP-K.

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 'Invulling definitie project' van de CCD. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. Het betreft explorerend basaal

wetenschappelijk onderzoek naar executief functioneren bij muizen. Er zijn twee primaire doelen in het project beschreven, die elk bijdragen aan de hoofddoelstelling. De DEC is er 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.

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 doelcategorie is in overeenstemming met de hoofddoelstelling.

   Belangen en waarden

4. Het directe doel van het project is te achterhalen hoe de verschillende typen cellen uit het executieve netwerk bijdragen aan instrumenteel leren. Het uiteindelijke doel is bijdragen aan nieuwe behandelmogelijkheden voor mensen waarbij het executief functioneren door ziekte of een aandoening is aangetast. De aanvragers noemen schizofrenie en ernstige depressie als voorbeelden van ziekten waarbij het executief functioneren minder goed verloopt. De resultaten van dit onderzoek zijn bovendien relevant voor wetenschappers die cognitie bestuderen of hersenonderzoek doen met dieren of computermodellen. Het betreft zeer basaal wetenschappelijk onderzoek, dat nog op grote afstand van een eventuele toepassing staat. De DEC is daarom van mening dat er binnen dit project geen reële relatie is tussen het directe doel en het uiteindelijke doel. De commissie vindt dat het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld, omdat het kennis zal opleveren die veel wetenschappers kunnen gebruiken in hun fundamentele of translationele onderzoek.

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).
   Dit onderzoek is in de eerste plaats wetenschappelijk van belang, omdat het naar verwachting bijdraagt aan een vermeerdering van de kennis in dit onderzoeksveld. Het betreft fundamenteel strategisch onderzoek gericht op het verwerven van fundamentele inzichten en kennis. Dergelijk onderzoek is onmisbaar voor de voortgang van zowel fundamenteel als translationeel onderzoek. Voor patiënten is dit onderzoek dus ook slechts indirect en op de lange termijn van belang. Uiteindelijk zouden de verworven inzichten kunnen bijdragen aan de ontwikkeling van nieuwe interventies voor met name neurologische aandoeningen. Kunnen beschikken over adequate behandelingen voor ernstige hersenziekten, zoals schizofrenie en ernstige depressie, is van groot belang voor de samenleving.

6. De onderzoekers maken gebruik van genetisch gemodificeerde virale vectoren en transgene

dieren waarbij zij de nationale GGO-regels in acht nemen. Hierdoor is er geen sprake van belangwekkende milieueffecten.

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 commissie heeft de aanvrager verzocht meer inzicht te geven in de hypothesen die getest zullen worden en te beschrijven welke stappen hiervoor nodig zijn. Het antwoord van de aanvrager wordt vooral gegeven op het niveau van de proefopzet. Dit type onderzoek is vooral explorerend van aard, en er worden zeer veel data verzameld tijdens de metingen in de proefopzet. Met deze data kunnen meerdere hypothesen door verschillende onderzoekers van meerdere onderzoeksgroepen/instituten worden onderzocht. Deze onderzoekers zijn geïnteresseerd in neurale oscillaties en het optreden daarvan tijdens een eenvoudige leertaak. Uit de beschrijving van de proefopzet blijkt dat een muis telkens een nieuwe (instrumentele) leertaak krijgt die enkele minuten duurt. Dit is een goede opzet om dergelijke leertaken te onderzoeken. 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 wordt hoofdzakelijk bepaald door de stereotactische operatie, de hersenmanipulaties aan een dier bij bewustzijn, en de lange looptijd waardoor langdurige solitaire huisvesting nodig is. De commissie is van mening dat het cumulatief ongerief voor de dieren hierdoor kan oplopen tot maximaal ernstig ongerief.

12. De integriteit van het dier wordt aangetast door het aanbrengen van een ‘headpost’ waardoor de kop gefixeerd kan worden tijdens de metingen. Het dier wordt hierdoor buiten de meetopstelling

in lichte mate gehinderd in zijn normale gedrag. Het aanbrengen van de 'headpost' verandert ook het uiterlijk van het dier. Het vormt een zichtbaar bewijs van het feit dat het dier "instrumenteel" gebruikt wordt. Tijdens de metingen zal het dier verder de indruk krijgen dat hij zich in een vreemde wereld bevindt waarin hij zich kan verplaatsen in de richting van niet-natuurlijke objecten. Dit staat op het eerste gezicht erg ver af van de natuurlijke omgeving van het dier. Echter, het dier is hiertoe in staat door van nature aanwezige nieuwsgierigheid en leervermogen. Er zijn geen aanwijzingen dat dit het gedrag en de psyche van het dier negatief beïnvloedt.

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 ervaringen 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. De onderzoekers willen interacties tussen bepaalde hersengebieden en/of neuronen onderzoeken, en het effect van stimulatie van bepaalde neuronen of hersengebieden op het leergedrag analyseren. Dit kan niet bij mensen en kan ook nog niet goed zonder proefdieren onderzocht worden. De resultaten zullen wel bijdragen aan het maken van computermodellen die op termijn gebruikt kunnen worden om bepaalde onderzoeksvragen te kunnen beantwoorden.

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. De onderzoekers gebruiken nieuwe analyses, waardoor zij moeilijk kunnen voorspellen hoeveel dieren per groep nodig zijn om significante resultaten aan te kunnen tonen. Zij gaan daarom uit van 12 dieren per groep. Wanneer uit tussentijdse analyses blijkt dat minder dieren per groep nodig zijn, dan zullen zij dit aanpassen in hun proefopzet. De onderzoekers zullen veel data op meerdere tijdstippen of in meerdere situaties verzamelen van elk dier, zodat hiermee verschillende onderzoeksvragen beantwoord kunnen worden door de onderzoekers zelf of door collega's die toegang krijgen tot de data. Hierdoor wordt de wetenschappelijke opbrengst van de experimenten geoptimaliseerd en zijn mogelijkerwijs voor het beantwoorden van toekomstige onderzoeksvragen geen, of minder, dieren nodig.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. De experimenten kunnen niet met minder complexe dieren worden gedaan omdat de hersenfunctie en het gedrag van deze dieren te weinig overeenkomen met de humane situatie. De onderzoekers plaatsen en ontwerpen de 'headpost' en de elektrodehouder zodanig dat de dieren er zo min mogelijk last van hebben. Om de stress van solitaire huisvesting te verminderen zullen de dieren regelmatig onder supervisie met nestgenoten kunnen interacteren. Na vragen van de commissie is de maximale looptijd van de experimenten per dier gereduceerd van 16 maanden naar 10 maanden. De onderzoekers hebben berekend dat zij in totaal ongeveer 40 uur dienen te meten aan één dier (verdeeld over meerdere meetsessies) om wetenschappelijk betrouwbare data te verkrijgen. Verdere beperking van de maximale looptijd zou de kwaliteit van de data in gevaar kunnen brengen. 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. Dieren van beide geslachten zullen in gelijke mate ingezet worden.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om de hersenen 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 matige tot maximaal 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 er alles aan om het lijden van de dieren en het aantal dieren te beperken.
   Voor patiënten is dit onderzoek indirect en pas op de lange termijn van belang. Uiteindelijk zouden de verworven inzichten kunnen bijdragen aan de ontwikkeling van nieuwe interventies voor met name neurologische aandoeningen waarbij het executief functioneren is aangetast, zoals schizofrenie en ernstige depressie. De huidige behandelingen hebben bij het merendeel van de patiënten te weinig resultaat. Kunnen beschikken over adequate behandelingen voor ernstige hersenziekten is van groot belang voor de samenleving. Dit onderzoek is echter in de eerste plaats van belang, omdat het naar verwachting bijdraagt aan een vermeerdering van de kennis in dit onderzoeksveld. Het betreft fundamenteel strategisch onderzoek gericht op het verwerven van fundamentele inzichten en kennis. De inzichten uit dergelijk onderzoek zijn onmisbaar voor de voortgang van zowel fundamenteel als translationeel onderzoek. De commissie acht meer kennis over executief functioneren en de bijdrage die die kennis uiteindelijk zou kunnen leveren aan het ontwikkelen van nieuwe behandelstrategieën voor verschillende aandoeningen van de hersenen, van substantieel belang.

3. De DEC is overtuigd van het belang van de doelstellingen: achterhalen hoe de verschillende typen cellen uit het executieve netwerk bijdragen aan instrumenteel leren. De DEC is van mening dat het belang van het verwerven van nieuwe kennis over het executief functioneren en de bijdrage die dat kan leveren aan de voortgang in dit onderzoeksveld en andere onderzoeksvelden (doordat de beschikbaar gestelde data ook bruikbaar zijn voor andere onderzoekers) 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.

Centrale Commissie Dierproeven

12.

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Onze referentie
Aanvraagnummer
AVD103002017873
Bijlagen
1

13 MRT 2017

Datum 10 maart 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted],

Op 17 februari 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Interneurons, oscillations, and learning" met aanvraagnummer AVD103002017873. Wij hebben uw aanvraag beoordeeld.

Op 8 maart 2017 heeft u uw aanvraag aangevuld. Er is u gevraagd formuleringen in de Niet Technische Samenvatting te herzien en in de bijlage dierproeven de afwijkende huisvesting en ongeriefclassificatie te verduidelijken. U heeft de vragen beantwoord en de Niet Technische Samenvatting en de bijlage dierproeven 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 "Interneurons, oscillations, and learning" starten. De vergunning wordt afgegeven van 17 maart 2017 tot en met 16 maart 2022. Deze termijn is anders dan in uw aanvraag, omdat de looptijd van een project niet langer kan zijn dan 5 jaar.

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.

De beoordeling achteraf is vereist vanwege de ongeriefclassificatie ernstig.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie RU DEC gevoegd. Dit advies is opgesteld op 17 februari 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

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

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

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

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

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

Datum:
10 maart 2017
Aanvraagnummer:
AVD103002017873

Centrale Commissie Dierproeven
namens deze:

[redacted]

ir. G. de Peuter
Algemeen Secretaris

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 17 maart 2017 tot en met 16 maart 2022, voor het project "Interneurons, oscillations, and learning" met aanvraagnummer AVD103002017873, 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 Instantie voor Dierenwelzijn verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 17 februari 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 17 februari 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 8 maart 2017;
c Advies van dierexperimentencommissie d.d. 17 februari 2017, ontvangen op 17 februari 2017.
d De aanvullingen op uw aanvraag, ontvangen op 8 maart 2017

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 Description of procedures for surgery and tasks.				
	Muizen (Mus musculus) / 6 veschillende stammen zoals beschreven in bijlage 3.4.4.1	395	95% Ernstig 5% Matig	

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 maart 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.

Aanvraagnummer:
AVD103002017873

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:
AVD103002017873

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.

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.