	Inventaris Wob-verzoek W16-04s								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS2015228								
1	Aanvraagformulier				x		x	x	
2	DEC-advies				x		x	x	
3	Niet-technische samenvatting	x							
4	Projectvoorstel				x		x	x	
5	Bijlagen dierproeven				x		x	x	
6	Ontvangstbevestiging				x		x	x	
7	Factuurinformatie				x		x		
8	Advies CCD		x						x
9	Beschikking en vergunning				x		x	x	

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1.

27 AUG. 2015

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	[redacted]
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
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	Postdoc
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

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

(Titel) Naam en voorletters	[redacted] ☑ Dhr. ☐ Mw.
Functie	Onderzoeker in opleiding
Afdeling	[redacted]
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	Instantievoor Dierenwelzijn
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	instantievoordierenwelzijn@radboudumc.nl

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	25-09-2015
Einddatum	25-09-2020

3.2 Wat is de titel van het project?

[redacted]

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

Nieuwe inzichten in en behandeling van cocaine en methamphetamine verslaving

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 € 741,00 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, factuurinformatie

6 Ondertekening

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

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

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

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

Naam	[redacted]
Functie	Instantie voor dierenwelzijn
Plaats	Nijmegen
Datum	25-08-2015
Handtekening	[redacted]

DEC-advies

A. Algemene gegevens over de procedure

1. Aanvraagnummer 2015-0021
2. Titel van het project: [redacted]
3. Titel van de NTS: Nieuwe inzichten in en behandeling van cocaine en methamphetamine verslaving
4. Type aanvraag:
   - ☐ nieuwe aanvraag projectvergunning
5. Contactgegevens DEC:
   - Naam DEC: RUDEC
   - Telefoonnummer contactpersoon: [redacted], bereikbaar op maandag, dinsdag, en donderdag van 9:00 tot 15:00 uur
   - Mailadres contactpersoon: [redacted]
6. Adviestraject:
   - ☐ ontvangen door DEC: 24-06-2015
   - ☐ in vergadering besproken: 07-07-2015
   - ☐ geen termijnonderbreking
   - ☐ aanpassing aanvraag: nvt
   - ☐ anderszins behandeld: nvt
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen: n.v.t.
   - ☐ advies aan CCD: 24-08-2015
7. Eventueel horen van aanvrager: n.v.t.
   - Datum
   - Plaats
   - Aantal aanwezige DEC-leden
   - Aanwezige (namens) aanvrager
   - Strekking van de vraag / vragen
   - Strekking van het (de) antwoord(en)
   - Het horen van de aanvrager heeft geleid tot aanpassing van de aanvraag
8. Correspondentie met de aanvrager
   - Datum:
   - Strekking van de vragen:
   - Datum antwoord:
   - Strekking van de antwoorden:
9. Eventuele adviezen door experts (niet lid van de DEC): n.v.t.
   - Aard expertise
   - Deskundigheid expert
   - Datum verzoek
   - Strekking van het verzoek
   - Datum expert advies
   - Expert advies

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig.
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.

C. Beoordeling (inhoud):

1. Het project is:

    - ☐ uit wetenschappelijk oogpunt verantwoord

2. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.
3. De DEC onderschrijft het belang van de doelstelling, namelijk 'to reveal how s[redacted] contribute to the neurochemical and drug-taking effects of cocaine and methamphetamine. And in addition to test the potential therapeutic effects of a number of treatments[redacted]'. Het wordt ingeschat als een substantieel belang. Het onderzoek betreft weliswaar een risicovolle hypothese, maar daarbij dient in aanmerking te worden genomen dat dit bij fundamenteel onderzoek niet ongebruikelijk is en dat de kans op bruikbare uitkomsten, [redacted], zeer reëel is. De maatschappelijke problematiek van verslaving en de behoefte aan gerichte therapieën die aangrijpen op het mechanisme van de verslaving, vertegenwoordigen eveneens een substantieel belang.
4. De gekozen strategie en experimentele aanpak zijn wetenschappelijk verantwoord en kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De DEC acht deze onderzoeksgroep zeer competent op dit onderzoeksgebied. De gekozen aanpak leidt in elk geval tot meer inzicht in de werking van psychostimulantia.
5. Er is geen sprake van bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren.
6. Het ongerief als gevolg van de dierproeven is voor de meeste dieren realistisch ingeschat en geclassificeerd. Het ongerief wordt hoofdzakelijk bepaald door het in vivo meten van serotonine in bepaalde hersengebieden, het induceren van verslaving aan cocaïne of methamphetamine en de handelingen die nodig zijn om zelftoediening van verslavende stoffen mogelijk te maken. De DEC schat het ongerief als gevolg van de injecties, de benodigde operaties, de blootstelling aan psychostimulantia gedurende korte of langere tijd en onthouding hiervan, en het ondergaan van een gedwongen zwemtest waarin immobiliteit wordt gemeten in als matig. Het ongerief voor de dieren die beide operaties ondergaan wordt ingeschat als ernstig. De meeste dieren ondergaan een gedwongen zwemtest, hetgeen in dit geval matig ongerief veroorzaakt. De DEC is op de hoogte van BIJLAGE VIII bij de Richtlijn 2010/63, waarin sprake is van ernstig ongerief bij gedwongen zwemtests, en meent dat de Nederlandse frasering "test met gedwongen zwemsessies **of** oefeningen met uitputting als eindpunt" berust op een foutieve vertaling van "forced swim or exercise tests with exhaustion as the end-point". Uit de Engelse versie blijkt dat het er om gaat dat een gedwongen zwemtest **met uitputting als eindpunt**, dient te worden ingeschaald als ernstig ongerief. De DEC heeft uitvoerig gediscussieerd over de juiste inschaling van het ongerief veroorzaakt door gedwongen zwemsessies, en heeft advies ingewonnen bij een proefdierdeskundige (die de testen in het verleden heeft geobserveerd) omtrent de inschaling

van het ongerief van de zwemtesten zoals beschreven in deze vergunningaanvraag. Zonder de deskundigheid van de mensen die die lijst met voorbeelden hebben opgesteld in twijfel te willen trekken, is de DEC van mening dat niet voor elke diersoort en niet voor elke variant van de test gesteld kan worden dat een forced swim test tot uitputting leidt. Er is in de onderhavige test geen sprake van dat men het tijdstip waarop daadwerkelijk uitputting optreedt (als uitleesparameter) wil vaststellen. Het blootstellen van de rat aan de gedwongen zwemtest zoals beschreven in deze vergunningaanvraag (1e dag 15 minuten, 2e dag 5 minuten zwemmen) kan als matig ongerief worden ingeschat, omdat mag worden aangenomen dat een rat 15 minuten kan zwemmen zonder uitgeput te raken. Het cumulatief ongerief voor het beschreven project is daarom terecht ingeschat als ernstig voor de dieren die twee operaties ondergaan (ongeveer 20% van de dieren) en matig voor de rest van de dieren. De commissie is van mening dat het cumulatief ongerief voor de dieren die eenmaal zijn geopereerd en herhaaldelijke injecties ontvangen, door de onderzoekers ten onrechte is ingeschat op ernstig. Zij is van mening dat het cumulatief ongerief voor deze dieren matig is.

7. Er zijn geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen **vervangen.** De doelstelling van het project kan niet gerealiseerd worden zonder proefdieren of door gebruik van minder complexe diersoorten.
8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de **vermindering** van dierproeven. De verschillende experimenten in het project volgen logisch op elkaar en een volgend experiment wordt pas gestart indien de resultaten van het voorafgaande experiment daartoe aanleiding geven. Het aantal benodigde dieren wordt geoptimaliseerd door resultaten uit voorgaande experimenten te gebruiken bij de opzet van de vervolgexperimenten. Het maximale aantal te gebruiken dieren is realistisch ingeschat en proportioneel ten opzichte van de gekozen strategie en de looptijd. De DEC is het eens met het beschreven onderzoeksmodel en de statistische onderbouwing van het aantal benodigde dieren. De DEC is van oordeel dat het project kan worden uitgevoerd met maximaal 2080 mannelijke Wistar ratten.
9. Het project is in overeenstemming met de vereiste van de **verfijning** van dierproeven. Bij de opzet wordt rekening gehouden met dierenwelzijn door toepassing van goede pijnbestrijding en anesthesie. De beschreven zwemtest is noodzakelijk voor het beantwoorden van de onderzoeksvraag en leidt in deze vorm tot niet meer dan matig ongerief. De DEC is ervan overtuigd dat de dierproeven zo humaan mogelijk worden uitgevoerd.
   Er is geen sprake van belangwekkende milieueffecten.
10. De niet-technische samenvatting is een evenwichtige weergave van het project, zelfstandig leesbaar, beknopt en begrijpelijk geformuleerd.

D. Ethische afweging

Op basis van de onder C genoemde overwegingen komt de DEC tot de volgende ethische afweging.

Met dit onderzoek worden belangrijke wetenschappelijke inzichten verworven in de cellulaire mechanismen die ten grondslag liggen aan het ontstaan van verslaving(sgedrag). Het is aannemelijk dat die inzichten kunnen bijdragen aan het ontwikkelen van therapeutische interventies bij verslaving. Het belang van meer inzicht in het ontstaan van verslaving en het beschikbaar komen van meer therapeutische opties acht de DEC substantieel, gezien de omvang van de maatschappelijke en individuele problematiek.

Tegenover dit substantiële belang staat het gegeven dat 80% van dieren die in dit onderzoek gebruikt worden matig ongerief en 20% van de dieren ernstig ongerief zullen ondervinden als gevolg van het *in vivo* meten van serotonineconcentraties in bepaalde hersengebieden, het induceren van cocaïne of methamphetamine verslaving en de handelingen die nodig zijn om zelftoediening van verslavende stoffen mogelijk te maken. De commissie is er van overtuigd dat bij de dierproeven adequaat invulling gegeven zal worden aan de vereisten op het gebied van de vervanging, vermindering en/of verfijning van dierproeven. Het gebruik van de dieren en het daarbij optredende ongerief is onvermijdelijk, wil men de doelstellingen kunnen realiseren.

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 onder de volgende voorwaarden
  - 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

2. Het uitgebrachte advies is gebaseerd op consensus.

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.	[redacted]

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.

Acute administration of cocaine and methamphetamine is known to drastically increase the extracellular levels of the monoamine serotonin in the brain [redacted] In-vitro studies have shown that cocaine may inhibit the neuronal re-uptake of monoamines by blocking plasmalemmal serotonin re-uptake transporters. However, on the basis of a series of in-vivo studies we have recently proposed that cocaine may also [redacted] monoaminergic storage vesicles ([redacted]). To what extend this novel [redacted] action of psychostimulants is depending on the [redacted] is currently unknown.

- In the **first experiment** we would like to investigate whether cocaine and methamphetamine are able to increase the extracellular levels of serotonin in the central amygdala of rats [redacted]

- In the **second experiment** we would like to establish whether cocaine and methamphetamine are able to [redacted] of the central amygdala in our [redacted] rats.

Cocaine and methamphetamine are highly addictive and have repeatedly been shown to result in voluntary self-administration in rats ([redacted]). Rats that are exposed to short (1h) daily sessions of cocaine or methamphetamine self-administration are marked by a moderate and stable psychostimulant intake, whereas the psychostimulant intake in rats that are exposed to long (6h) daily sessions of cocaine or methamphetamine self-administration has been found to strongly increase ([redacted]). This 'long access induced escalation of the drug intake' is generally accepted to model the transition from 'normal' to 'compulsive' drug use observed in human addicts.

In human addicts a genetic deletion of [redacted] has been found to increase the self-administration of psychostimulants ([redacted]- [redacted] Interestingly, an increase of the short access psychostimulant intake has also been found in our [redacted] rats

([redacted]). Given the previously reported action of psychostimulants on the re-uptake of serotonin ([redacted] is also expected to affect the long access drug intake. The proposed action of cocaine and methamphetamine on the [redacted]), suggests that [redacted] may also lead to changes in the short and/or long access intake of these drugs.

- In the **third experiment** we would like to study cocaine and methamphetamine self-administration in [redacted] rats under both short and long access conditions.

At this point we don't know whether the increased psychostimulant intake in subjects with a [redacted]s due to a [redacted] during adulthood or during development. This information is vital to establish when during life future serotonergic treatment strategies should be applied (see experiment 6 below).

- In the **fourth experiment** we would like to study cocaine and methamphetamine self-administration under short and long access conditions in rats in which [redacted] only during adulthood. The psychostimulant intake of these commercially available so called [redacted] ([redacted] will be compared to the psychostimulant intake of our own '[redacted]', in which [redacted] during their whole life (see experiment 3).

Serotonergic neurons are known to arise either in the dorsal or median raphe nuclei. At this point it is unknown which of these brains regions mediates the increased psychostimulant intake in subjects with [redacted] However, preliminary results have shown that [redacted] in neurons arising from the dorsal, but not the median, raphe increase the long access intake of cocaine ([redacted]).

- In the **fifth experiment** we would like to stimulate or inhibit the serotonergic neurons of the dorsal raphe of adult [redacted] rats to investigate whether [redacted] in the neurons arising in this brain region contributes to cocaine and methamphetamine self-administration under both short and long access conditions. The type of [redacted] animals to be used in this experiment (constitutive or conditional) depends on the results of experiment 4 ([redacted]). The serotonergic neurons of the dorsal raphe are stimulated or inhibited using optogenetic techniques in collaboration with [redacted] In case optogenetic manipulation of the serotonergic neurons of the dorsal raphe does not alter psychostimulant intake, a new DEC proposal will be submitted seeking permission to test the effects of the optogenetic manipulation of the median raphe neurons of our rats.

The previously reported finding that [redacted] changes psychostimulant self-administration (see above) indicates that drugs that alter extracellular serotonin levels may potentially have therapeutic effects in the treatment of both the normal and the compulsive intake of cocaine and methamphetamine (see also: [redacted]).

- In the **sixth experiment** we would like to test how changing the extracellular levels of serotonin alters cocaine self-administration under short and long access conditions in both [redacted] rats. Given that changing extracellular serotonin may have different effects during development versus adulthood (see experiment 4), we aim to expose our rats to the potentially therapeutic drugs directly after birth or during the self-administration experiment that takes places at adulthood. The type of [redacted] animals to be used in this experiment ([redacted]) depends on the results of experiment 4 ([redacted]).

Exposure to various changes in the environment during development has been found to alter the central levels of serotonin as well as psychostimulant intake (for review: [redacted]). This suggests that the serotonergic make-up and reactivity of the brain of our [redacted] rats makes these animals more susceptible to the potentially beneficial effects of environmental enrichment on psychostimulant intake.

- In the **seventh, and final, experiment**, we would like to test how environmental enrichment during development changes cocaine and methamphetamine self-administration in our [redacted] and [redacted] rats under both short and long access conditions. Combining the results of the final 2 experiments will reveal whether addicted individuals with a [redacted] or [redacted] may benefit only from pharmacological treatments (experiment 6), or also from potentially less invasive changes in the environment (experiment 7). The type of [redacted] animals to be used in the final experiment ([redacted]) depends on the results of experiment 4 ([redacted] 4, constitutive [redacted] rats are preferred).

References

- Ahmed SH, Koob GF (1998). Transition from moderate to excessive drug intake: change in hedonic set point. *Science* **282:** 298-300.
- Enoch MA, Gorodetsky E, Hodgkinson C, Roy A, Goldman D (2011). Functional genetic variants that increase synaptic serotonin and 5-HT3 receptor sensitivity predict alcohol and drug dependence. *Mol Psychiatry* **16:** 1139-1146.
- Gerra G, Zaimovic A, Garofano L, Ciusa F, Moi G, Avanzini P *et al* (2007). Perceived parenting behavior in the childhood of cocaine users: relationship with genotype and personality traits. *Am J Med Genet* **144B:** 52-57.
[redacted]
- Kitamura O, Wee S, Specio SE, Koob GF, Pulvirenti L (2006). Escalation of methamphetamine self-administration in rats: a dose-effect function. *Psychopharmacology (Berl)* **186:** 48-53.
- Martin-Santos R, Torrens M, Poudevida S, Langohr K, Cuyas E, Pacifici R *et al* (2010). 5-HTTLPR polymorphism, mood disorders and MDMA use in a 3-year follow-up study. *Addict Biol* **15:** 15-22.
[redacted]
- Oakly AC, Brox BW, Schenk S, Ellenbroek BA (2014). A genetic deletion of the serotonin transporter greatly enhances the reinforcing properties of MDMA in rats. *Mol Psychiatry* **19:** 534-535.
[redacted]

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 aim of the suggested experiments is to reveal how [redacted] contribute to the neurochemical and drug-taking effects of cocaine and methamphetamine. In addition, we would like to test the potential therapeutic effects of a number of treatments that are known to change the [redacted]. We seek permission to perform a series of 7 experiments. Based on our substantial experience with the suggested experiments (stereotactic surgery, microdialysis and [redacted] measurements: see a.o. [redacted] IV surgery and short + long access self-administration: [redacted]'; IV surgery, self-administration + serotonergic manipulations: see a.o. [redacted]; Porsolt swim test + serotonergic manipulations: see a.o. H[redacted] and optogenetics: see [redacted]) and the planning of these experiments, we believe it will take us 5 years to complete the whole study.

Note regarding feasibility: three additional project members have been added to assist during the experiments. These scientists will receive a thorough training by the responsible researcher. In addition, the research team can always rely on the knowledge and advice of various experts in the fields.

3.3 Relevance

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

Medical significance:
At this moment there is no FDA approved pharmacological treatment option for psychostimulant addiction. The aim of the proposed experiments is to study the cellular mechanisms underlying the rewarding and addictive effects of both cocaine and methamphetamine. Finding a novel working mechanism for the behavioral effects of these two psychostimulants and testing the putative therapeutic effects of a number of drugs that interfere with these mechanisms may in the future lead to the development of a highly desired pharmacological treatment for cocaine and methamphetamine dependence.

Scientific significance:
Our experiments will help to understand why human subjects marked [redacted] of extracellular serotonin as well as [redacted] in the psychostimulant-induced increase of the extracellular levels of this monoamine have an increased risk to become addicted. Finding an answer to this question is particularly relevant because at least 20% of the human population is marked by this serotonergic make-up of the brain. In addition, our experiments may reveal an important role for serotonin in the switch from moderate to compulsive psychostimulant intake.

Societal significance:

Drug dependence causes a large number of very unpleasant clinical symptoms (e.g. psychosis during the drug intake and anxiety and depression when the patient is in withdrawal). It may not only affect the patent's life, but also that of his/her family members and friends. In addition, drug addiction leads to high medical and economical expenses. In the US drug abuse accounts for more than $500 billion per year associated to health care, productivity loss, crime, law enforcement and incarceration. Therefore, studying the working mechanisms of the behavioral effects of cocaine and methamphetamine and testing a number of novel treatment options for psychostimulant addiction may in the future have beneficial effects not only for addicted patients, but also for the other members of our society.

In summary, we believe that the medical, scientific and societal significance of our study outweighs the potential discomfort the animals may be exposed to.

3.4 Research Strategy

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

The project consists of the 7 experiments that are listed below.

We seek permission to perform our studies in [redacted] rats because [redacted]

Aims and details can be found in sections 3.1 (Background) and 3.4.2 (Research outline) respectively.

1a Microdialysis [redacted] rats
1b Microdialysis [redacted] rats

- Drugs and delivery: systemic injection of cocaine and methamphetamine.
- Readout parameter: extracellular serotonin levels in the central amygdala of rats [redacted] during both development and adulthood.
- We propose to perform microdialysis because it is a reliable, sensitive, and relatively easy technique to directly measure changes in the extracellular levels of serotonin in the brain. The advantage of the suggested technique above other techniques, like for instance immunohistochemistry, is that one doesn't need to sacrifice a large number of animals to measure the changes in serotonin over time. In addition, serotonin measurement can be performed in freely moving animals. Moreover, we have substantial experience with the suggested technique (e.g. [redacted]

2a [redacted] release [redacted] rats
2b [redacted] release [redacted] rats

- Drugs and delivery: systemic injection of cocaine and methamphetamine.

- Readout parameter: [redacted] in the central amygdala of rats that are lacking [redacted] or [redacted] during both development and adulthood.
- We propose to measure [redacted] according to our previously reported procedures ([redacted]). Compared to electronmicroscopy, our technique is faster, more sensitive, cheaper and most importantly more quantitative.

3a Self-administration [redacted] rats
3b Self-administration [redacted] rats

- Drugs and delivery: voluntary cocaine and methamphetamine self-administration under short and long access conditions.
- Readout parameter: number of active lever presses in rats that are lacking [redacted] or [redacted] during both development and adulthood.
- Self-administration is the most used technique to measure the intake of drugs of abuse in animals. It has high face validity to the human situation and is perfect to test the effects of anti-addiction treatments. Compared to systemic injections of drugs of abuse, in the self-administration procedures the intake of these drugs in voluntary and less stressful (no injections). The [redacted]

4a Self administration [redacted] rats
4b Self administration [redacted] rats

- Drugs and delivery: voluntary cocaine and methamphetamine self-administration under short and long access conditions.
- Readout parameter: number of active lever presses in rats that are lacking [redacted] or [redacted] during adulthood only.
- We propose to use [redacted] rats because in these animals, in contrast to [redacted] animals, we can decide ourselves when the gene of interest is turned on and when it is off.

5a Self-administration [redacted] rats with optogenetically [redacted] serotonin
5b Self-administration [redacted] rats with optogenetically [redacted] serotonin

- Drugs and delivery: voluntary cocaine and methamphetamine self-administration under short and long access conditions.
- Readout parameter: number of active lever presses under short and long access conditions in [redacted] or [redacted] rats of which the activity of the dorsal raphe nucleus neurons is manipulated.
- In contrast to [redacted] rats, in which the activity of the serotonergic neurons is changed throughout the brain, 'optogenetic rats' can be used to change the activity of the serotonergic neurons in one particular brain region only. Experiment 5 will be performed in collaboration with prof. dr. [redacted] who has extensive experience with the proposed technique.

6a Self-administration [redacted] rats with [redacted] at adulthood
6b Self-administration [redacted] rats with pharmacologically [redacted] serotonin at adulthood
6c Self-administration [redacted] rats with [redacted] during development
6d Self-administration [redacted] rats with pharmacologically [redacted] serotonin during development

- Drugs and delivery: voluntary cocaine and methamphetamine self-administration under short and long access conditions.

- Readout parameter: number of active lever presses under short and long access conditions in [redacted] or [redacted] rats that are treated with drugs known to [redacted] are proposed because they have already been found in other studies to change the brain levels of serotonin.

7a Self-administration [redacted] rats after environmental enrichment
7b Self-administration [redacted] rats after environmental enrichment

- Drugs and delivery: voluntary cocaine and methamphetamine self-administration under short and long access conditions.
- Readout parameter: number of active lever presses under short and long access conditions in [redacted] or [redacted] rats that are exposed to an enriched environment.
- Environmental changes have in other studies already proven to change the behavior of our [redacted] rats.

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

Experiment 1a: Microdialysis in [redacted] rats / Experiment 1b: Microdialysis in constitutive [redacted] rats

Male rats will be implanted with a guide cannula in the brain under isoflurane anesthesia. After a recovery period of at least 7 days, a microdialysis probe is [redacted] into the guide cannula to measure the extracellular levels of serotonin using high-performance liquid chromatography equipment which is coupled to an electrochemical detector. 4 hours after probe [redacted] the rats receive a single injection of cocaine, methamphetamine or saline. Extracellular serotonin levels and behavior are recorded for 4 more hours. After this microdialysis experiment, the brains of the animals are collected using a guillotine.

Experiment 2a: [redacted] release in [redacted] rats / Experiment 2b: [redacted] release [redacted] rats

Male rats will receive a single injection of cocaine, methamphetamine or saline. After a period of 20 minutes, during which behavior is recorded, the brains of the animals are collected using a guillotine. The brain regions of interest are punched out and [redacted]

Experiment 3a: Self-administration [redacted] rats / Experiment 3b: Self-administration [redacted] rats

Male rats will be equipped with a catheter in the jugular vein under isoflurane anesthesia. The tip of the catheter is secured to a cannula that is fixed to the back of the animal. This cannula will be connected to a syringe, which delivers an intraveneous injection of cocaine or methamphetamine every time the animal presses a lever. To model moderate psychostimulant intake, rats will be daily exposed to the self-administration chamber for 1 hour only (short access group). To study the compulsive intake of cocaine and methamphetamine, new rats will be exposed to the self-administration chambers for 6 hours per day (long access group).

Rats will be exposed to psychostimulant self-administration chambers for 28 days (10 days of 1-hour-lasting training sessions + 18 consecutive days of short and long access exposure to cocaine or methamphetamine), whereafter the animals are tested for 2 more days in the Porsolt swim test

(daily placement of the rat into a glass cylinder filled with water). After this behavioral test that measures the animal's emotional (i.e. depression-like) state, rats are placed again in the self-administration chambers while cocaine or methamphetamine is replaced by saline. The resulting extinction of psychostimulant seeking is measured for an additional period of 10 days. After this extinction period, the re-instatement of psychostimulant intake will be studied for a final period of 5 consecutive days. 24 Hours after the last re-instatement session, the brains of the animals are collected using a guillotine.

Experiment 4a: Self-administration [redacted] rats / Experiment 4b: Self-administration in [redacted] rats

Male rats of which [redacted] or [redacted] turned off only during development will be equipped with a catheter in the jugular vein according to the procedures described in experiment 3. After recovery, these rats will be trained for cocaine or methamphetamine self-administration for 10 days, whereafter the animals will be exposed to either short or long access sessions of psychostimulant intake for an additional period of 18 consecutive days. Similar to the previous experiments, the animals will also be tested for 2 days in the Porsolt swim test + 10 days of extinction and 5 consecutive days of re-instatement. 24 Hours after the last re-instatement session, the brains of the animals are collected using a guillotine.

Remark: The psychostimulant intake of these [redacted] rats will be compared to the psychostimulant intake observed [redacted] rats of experiment 3, in which [redacted] or [redacted] during both development and adulthood.

Experiment 5a: Self-administration in [redacted] rats with optogenetically [redacted] serotonin
Experiment 5b: Self-administration in [redacted] rats with optogenetically [redacted] serotonin

Male [redacted] or [redacted] rats will be equipped with a catheter in the jugular vein according to the procedures described in experiment 3. After 10 days of cocaine or methamphetamine self-administration training, these rats will be injected with viral vectors [redacted] under the influence of light (optogenetic i[redacted] of dorsal raphe nucleus (DRN) neurons). These viral vectors will be locally applied using the surgery procedures similar to the procedures described in experiment 1. After 1 week of recovery + 2 more weeks of viral vector incubation, rats will be exposed to either short or long access psychostimulant self-administration for an additional period of 18 consecutive days. Similar to the previous experiments, the animals will also be tested for 2 days in the Porsolt swim test + 10 days of extinction and 5 consecutive days of re-instatement. 24 Hours after the last re-instatement session, the brains of the animals are collected using a guillotine.

Remark: the group of rats to be used [redacted] is depending on the results of experiments 3 and 4. Because [redacted], [redacted] rats are preferred above [redacted] rats, unless the first group of rats does not display a change in psychostimulant self administration.

Experiment 6a: Self-administration [redacted] rats with [redacted] at adulthood
Experiment 6b: Self-administration [redacted] rats with pharmacologically [redacted] serotonin at adulthood
Experiment 6c: Self-administration [redacted] rats with [redacted] during development
Experiment 6d: Self-administration [redacted] rats with pharmacologically [redacted] serotonin during development

In experiments 6c and 6d, male rats that had access to respectively a [redacted] enriched diet after birth will be equipped with a catheter in the right jugular vein according to the procedures described in experiment 3. After recovery, these rats will be trained for cocaine or methamphetamine self-administration for 10 days, whereafter the animals will be exposed to either short or long access sessions of psychostimulant intake for an additional period of 18 consecutive days. Similar to the previous experiments, the animals will also be tested for 2 days in the Porsolt swim test + 10 days of extinction and 5 consecutive days of re-instatement. 24 Hours after the last re-instatement session, the brains of the animals are collected using a guillotine.

In experiments 6a and 6b, before the 5 final short or long access psychostimulant self-administration sessions, as well as before the second Porsolt swim tests and the final 2 re-instatement sessions, treatment-naive male rats will be systemically treated with ([redacted] of extracellular serotonin respectively. On the first treatment day, rats will receive the solvent of these drugs ([redacted]

Remark: the group of rats to be used to pharmacologically reduce extracellular serotonin is depending on the results of experiments 3 and 4. Because of financial reasons, constitutive [redacted] rats are preferred above conditional [redacted] rats, unless the first group of rats does not display a change in psychostimulant self-administration.

Experiment 7a: Self-administration [redacted] rats after environmental enrichment
Experiment 7b: Self-administration [redacted] rats after environmental enrichment

Male rats that grew up in an enriched environment (including 'catheter-proof' climbing, chewing, shredding, pushing and/or foraging toys) will be equipped with a catheter in the jugular vein according to the procedures described in experiment 3. After recovery, these rats will be trained for cocaine or methamphetamine self-administration for 10 days, whereafter the animals will be exposed to either short or long access sessions of psychostimulant intake for an additional period of 18 consecutive days. Similar to the previous experiments, the animals will also be tested for 2 days in the Porsolt swim test + 10 days of extinction and 5 consecutive days of re-instatement. 24 Hours after the last re-instatement session, the brains of the animals are collected using a guillotine.

Remark: the group of rats to be used to [redacted] is depending on the results of experiments 3 and 4. [redacted] financial reasons, [redacted] rats are preferred above [redacted] rats, unless the first group of rats does not display a change in psychostimulant self-administration.

Note: more details on the experimental procedures can be found in the 'animal procedure' tab, section A2.

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

Combining the results of the suggested experiments is expected to provide vital information with respect to the role o[redacted] in both the neurochemical and drug-taking effects of cocaine and methamphetamine. The first 2 experiments are designed to analyze the cellular mechanisms underlying the acute psychostimulant-induced increase of extracellular serotonin. The results of experiments 1 and

2 (milestone 1) will be used to select the drug(s) of abuse (cocaine and/or methamphetamine) and the subjects (and/or to be tested in the remaining experiments. This means that the more invasive and time consuming cocaine and methamphetamine self-administration experiments 3 and 4 will be performed only in case experiments 1 and/or 2 show that the genetic deletion of or affects the neurochemical effects of cocaine or methamphetamine. The results of self-administration experiments 3 and 4 (milestone 2) are, in turn, crucial to select the type of rats (to be used in experiments 5, 6 and 7 (milestone 3). This means that the final brain region specificity studies (experiment 5), the pharmacological manipulations (experiment 6) and the environmental enrichment experiments (experiment 7) will be performed only in case experiments 3 and/or 4 show of or changes the number of levers presses to obtain cocaine or methamphetamine.

By using this from neuron to behavior 'go / no go' strategy, we are able to adjust the number of animals to be tested to an absolute minimum. Depending on the results, we will need to use between 288 (only experiments 1 and 2) and 2080 (all experiments) rats (see section for details). If all 7 experiments are successful, we will need 5 years to complete the suggested work.

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	Experiments 1-2: microdialysis + release
2	Experiments 3-7: self-administration

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 Experiments 1-2: microdialysis + [redacted] release

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.

Microdialysis in [redacted] (exp 1a) and [redacted] (exp 1b) rats

Using microdialysis, extracellular serotonin levels are measured after a single systemic dose of cocaine (presumably 15 mg/kg) or methamphetamine (presumably 1.5 mg/kg) in [redacted] and [redacted] (exp 1b) rats. [redacted] Combining the results of the suggested experiments we aim to answer the important question whether the two psychostimulants under investigation increase the synaptic levels of serotonin because of [redacted], [redacted]. Neurotransmitters are collected using a dialysis probe [redacted] into the brain region of interest and serotonin is separated from the remaining neurotransmitters using high performance liquid chromatography (HPLC), whereafter its concentration will be measured using electrochemical detection (ECD).

[redacted] release in [redacted] and [redacted] ([redacted] rats

An additional group of [redacted] rats as well as an extra group of [redacted] rats will also receive a single systemic injection of the two psychostimulants (presumable doses: see above), [redacted]

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

Stereotactic surgery (exp 1a/b):

- Male rats will be implanted with a guide cannula in the brain (surgery time: approximately 45-60 minutes). Stereotactic surgery will be performed under isoflurane anesthesia. Lidocaine spray will also be applied before the periosteum is removed. Between the different surgeries, tools will be sterilized using a beat sterilizer and the skin of the animal will be disinfected using iodine and alcohol pads. Possible bleeding will be stopped using epinephrine pellets. In order to stay focused small breaks are taken after the surgery of every 2 rats and no more than 8 rats will undergo surgery per day. Immediately after surgery, rats will be injected with an analgesic drug (probably: Flunixin). Rats will only be moved to their animal room at 1 hour after surgery. During this period, the wound, breathing and activity of the animal is checked every 15 minutes. During the at least 7-days-lasting recovery time, the condition of the animals will carefully be monitored (water consumption, weight, behavior, condition of the wound, condition of the fur) and rats will be treated once daily with an antibiotic drug (probably: Cefazolin).

Microdialysis (exp 1/b): start between 8 and 9 am

- After the recovery period, a microdialysis probe is inserted into the guide cannula to measure the extracellular levels of serotonin using high performance liquid chromatography (HPLC) equipment which is coupled to an electrochemical detector (ECD).

Psychostimulant-induced behavior (exp 1a/b, 2a/b): start between 12 and 13 pm

- Rats receive a single injection of cocaine (presumable dose: 15 mg/kg), methamphetamine (presumable dose: 1.5 mg/kg) or saline (presumable volume: 1 ml/kg) and behavior is recorded for an additional period of 4 hours (exp 1/b) or 20 mins (exp 2a/b).

day 0: Decapitation (exp 1a/b, 2a/b):

- When the behavioral recordings are done, the brains of the animals are collected using a guillotine. These brains are used to measures the local changes in [redacted] (exp 1a/b) or the [redacted] levels of serotonin in the brain regions of interest (exp 2a/b).

Total duration of the various experiments:

- exp 1a/b: 1 day of surgery/recovery + 6 more days of recovery + 1 day of microdialysis = 8 days
- exp 2a/2b: 1 day (20 mins).

Justification of approach:

- We propose to perform microdialysis because it is a reliable, sensitive, and relatively easy technique to directly measure changes in the extracellular levels of serotonin in the brain. The advantage of the suggested technique above other techniques, like for instance immunohistochemistry, is that one doesn't need to sacrifice a large number of animals to measure the changes in serotonin over time. In addition, serotonin measurement can be performed in freely moving animals. Compared to electronmicroscopy, our technique to measure the [redacted] s faster, more sensitive, cheaper and, most importantly, more quantitative. The suggested procedures are based on previous experience ([redacted]). If minor changes have to be made, this will first be discussed with the institutional Welfare Officer (IVD). If necessary, an amendment will be submitted to the Central Authority for Scientific Procedures on Animals (CCD).

Note 1: Only the animals of experiment 1 will undergo surgery
Note 2: To ensure that, at any given time, test and control rats are exposed to the same conditions, the animals of every single experiment will be equally distributed across test and control groups.

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

The minimum number of animals to be used in every single experiment is calculated using a power analysis (see section B1 for details). After reaching a milestone (see section 3.4.3: coherence), data will first be analyzed and follow-up studies will only be performed if the previous studies have revealed promising results. By using this 'go / no go' approach (see also section 3.4.3: coherence) we will significantly reduce the risk of performing animal studies that should not have been performed.
Data will be statistically analyzed using an ANOVA with the factors genotype, treatment and time (exp 1a and 1b) and genotype and treatment (exp 2a and 2b).

B. The animals

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

The total maximum number of animals to be used in this animal procedure is 280. The animal origin is decribed in the Table.
Justification of the animals:

We seek permission to perform our studies in [redacted] rats because [redacted] are the main proteins regulating respectively the re-uptake and intra-[redacted] levels of serotonin. Only male rats will be used because fluctuations in the levels of stress hormones, associated with the menstrual cycle of female rats, may negatively affect the outcome of the suggested studies. The rats have to be adult to ensure that the volume of the central amygdala is maximal.

Treatment groups:

exp 1a: Microdialysis in [redacted] rats

[redacted] saline n=12, cocaine n=12, methamphetamine n=12
WT: saline n=12, cocaine n=12, methamphetamine n=12

exp 1b: Microdialysis in constitutive [redacted] rats

[redacted] saline n=12, cocaine n=12, methamphetamine n=12
WT: saline n=12, cocaine n=12, methamphetamine n=12

exp 2a: [redacted] release in [redacted] rats

[redacted] saline n=12, cocaine n=12, methamphetamine n=12
WT: saline n=12, cocaine n=12, methamphetamine n=12

exp 2b: [redacted] release in constitutive [redacted] rats

[redacted] [redacted] saline n=12, cocaine n=12, methamphetamine n=12
WT: saline n=12, cocaine n=12, methamphetamine n=12

Power analysis:

Previous studies using psychostimulant-treated knock-out animals have revealed a significant genotype effect when the effect-size is 50% (mean [redacted] is 150% of mean WT). Using this effect size, together with the obtained standard deviation and an alpha-value of 0.05 and a power of 0.80 revealed a minimum number of 10 rats per treatment group. Per treatment group 2 rats have been added to compensate for the animals that have to be excluded from our study because of incorrect placement of the microdialysis probe (exp 1a/b) or because of incorrect punching of the amygdala (exp 2a/b). If the number of animals has to be adjusted due to unforeseen circumstances, this will first be discussed with the institutional Welfare Officer (IVD). If necessary, an amendment will be submitted to the Central Authority for Scientific Procedures on Animals (CCD).

Species	Origin	Maximum number of animals	Life stage
exp 1a: Wistar [redacted] [redacted] + [redacted] WT	Own breeding program	72	Adult
exp 1b: Wistar [redacted] [redacted] + [redacted] WT	Own breeding program	72	Adult
exp 2a: Wistar [redacted] [redacted] + [redacted] WT	Own breeding program	72	Adult
exp 2b: Wistar [redacted] [redacted] + [redacted] WT	Own breeding program	72	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:

To answer our research questions we need to study the neurochemical changes after cocaine or methamphetamine in the brains of animals with a reduced expression of either [redacted] or [redacted] For this we use our unique [redacted] knock-out rats. Human subjects cannot be used because many people taking psychostimulants do also abuse other 'serotonin-changing' drugs like nicotine, alcohol and/or opiates. In addition, the amount of psychostimulant intake and the time between final drug use and the collection of brain tissue cannot be controlled in human subjects. Lower animals cannot be used because there is no microdialysis equipment available (invertebrates) or the surgery procedures are relatively complicated (mice) leading to an unacceptable high exclusion of animals (i.e. mice in which the position of the probe or punch was wrong). Cell-lines cannot be used because we need link neurochemical changes to changes in behavior. From our previous studies we know that in rats microdialysis is relatively easy to perform, resulting in reliable results.

Reduction:

We are using the absolute minimum number of animals necessary to still be able to discover potentially statistical significant differences between the various genotypes and/or treatments. Using even less animals leads to an increase of the standard error of mean (sem), thereby reducing the statistical power.

Refinement:

See section D2 below.

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

The discomfort our rats will be exposed to is limited to the absolute minimum necessary to answer our research questions. Surgery will be performed under isoflurane anesthesia. In addition, Lidocaine spray will be applied to the periosteum and the skin of the head. Possible bleeding will be stopped using epinephrine pellets. In order to stay focused, small breaks are taken after the surgery of every two rats and no more than 8 rats will undergo surgery per day. After surgery, rats will be treated with Flunixin (analgesic drug) and Cefazolin (antibiotic drug). Rats will only be moved to their animal room at 1 hour after surgery. During this period, the wound, breathing and activity of the animal is checked every 15 minutes. During the recovery time of at least 1 week, the animal's condition will be monitored on a daily basis. All animals will be handled to familiarize them to the experimental procedures and cage enrichment, in the form of a small wooden block and nest material, will also be available.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

Accommodation and care

F. Accommodation and care

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

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

At least 2 weeks before surgery (exp 1a and 1b) or the behavioral measurements (exp 2a and 2b), rats will be housed in groups of 2 or 3, in an animal room with a reversed day/night cycle and shelters will be provided. The rats that have undergone surgery (exp 1a and 1b) will be singly housed. Cagemates are removed because they will damage the cannula. Given that a collision with the sharp edges of a shelter may result in the loss of the cannula, shelters are replaced by wooden blocks and nest material. Rats are housed under a reversed day/night cycle to make sure that they will show the largest possible repertoire of different behaviors when they are tested.

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.

I. Other aspects compromising the welfare of the animals

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

Due to stress after surgery, rats may be more afraid to human contact. However, after 3 days of handling, these rats show normal behavior again. The animal's welfare may also be affected by the lack of a shelter or cagemates.

Explain why these effects may emerge.

Surgery, recovery from surgery, removal of shelters and cagemates.

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

The discomfort our rats will be exposed to is limited to the absolute minimum necessary to answer our research questions. Surgery will be performed under isoflurane anesthesia. In addition, Lidocaine spray will be applied to the periosteum and the skin of the head. Possible bleeding will be stopped using epinephrine pellets. In order to stay focused, small breaks are taken after the surgery of every two rats and no more than 8 rats will undergo surgery per day. After surgery, rats will be treated with Flunixin (analgesic drug) and Cefazolin (antibiotic drug). Rats will only be moved to their animal room at 1 hour after surgery. During this period, the wound, breathing and activity of the animal is checked every 15 minutes. During the recovery time of at least 1 week, the animal's condition will be monitored on a daily basis. All animals will be handled to familiarize them to the experimenter and the experimental procedures and cage enrichment, in the form of a small wooden block and nest material, will also be available.

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.

If the animal looses its cannula or when the bodyweight after surgery stays below 85% of the pre-surgery weight for 3 consecutive days, animals will be euthanized using CO2. In case of unexpected changes in the animal's behavior (e.g. no self-grooming or no water intake), or when the animal does not respond to a stimulus anymore, it will also be sacrificed using CO2.

Indicate the likely incidence.

Previous studies have shown that less than 5% of the animals reach their humane endpoint.

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).

exp 1a/1b: moderate, exp 2a/2b: mild

End of experiment

L. Method of killing

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.

Rats will be sacrificed using a guillotine. Brains will be used to locate the microdialysis probe track (exp 1a/1b) and to measure [redacted] levels of serotonin (exp 2/b) as well as gene expression levels (exp 1a/1b and 2a/2b).

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

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

[X] Yes

Appendix
Description animal procedures

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure Experiments 3-7: self-administration

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.

Cocaine and methamphetamine self-administration (exp 3, 4, 5, 6 and 7) in [redacted] (a) and [redacted] (b) rats

Using the self-administration paradigm, the voluntary intake of cocaine (presumable dose: 0.5 mg/kg/infusion) or methamphetamine (presumable dose: 0.05 mg/kg/infusion) will be established in [redacted] rats. [redacted] rats lack plasmalemmal serotonin re-uptake transporters and [redacted] rats lack an important enzyme to synthesize and store serotonin. Combining the results of the suggested experiments we aim to answer the important question whether serotonin re-uptake inhibition and/or the release of serotonin from presynaptic storage pools contribute to psychostimulant addiction. Rats will be exposed to either daily 1h sessions or 6h sessions of psychostimulant self-administration. The short access paradigm models limited drug intake, whereas the long access paradigm models drug dependence.

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

IV surgery (exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b):

- Male rats will be equipped with a catheter in the jugular vein under isoflurane anesthesia (surgery time: approximately 30-45 minutes). The tip of the catheter is secured to a cannula that is fixed to the back of the animal. In addition, lidocaine spray will be applied to the skin. Between the different surgeries, tools will be sterilized using a beat sterilizer and the skin of the animal will be disinfected using iodine and alcohol pads. Possible bleeding will be stopped using epinephrine pellets. In order to stay focused small breaks are taken after the surgery of every 3 rats and no more than 12 rats will undergo surgery per day. Immediately after surgery, rats will be injected with an analgesic drug (probably: Flunixin). Rats will only be moved to their animal room at 1 hour after surgery. During this period, the wound, breathing and activity of the animal is checked every 15 minutes. During the at least 7-days-lasting recovery time, the condition of the animals will carefully be monitored (water consumption, weight, behavior, condition of the wound, condition of the fur) and rats will be intraveneously treated once daily with a mix of Heparin (anticoagulant) and an antibiotic drug (probably: Cevazolin).

Self-administration training (exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b):

- After the recovery period, the IV cannula will be connected to a syringe, which delivers an intraveneous injection of cocaine (presumable dose: 0.5 mg/kg/infusion) or methamphetamine (presumable dose: 0.05 mg/kg/infusion) every time the animal presses a lever. Psychostimulant self-administration training (1 h daily session) takes 10 days. [redacted]

Stereotactic surgery (exp 5a/b):

- Rats will be implanted with a guide cannula in the dorsal raphe nucleus (surgery time: approximately 45-60 minutes). Stereotactic surgery will be performed under isoflurane anesthesia. Lidocaine spray will also be applied before the periosteum is removed. Between the different surgeries, tools will be sterilized using a beat sterilizer and the skin of the animal will be disinfected using iodine and alcohol pads. Possible bleeding will be stopped using epinephrine pellets. In order to stay focused small breaks are taken after the surgery of every 2 rats and no more than 8 rats will undergo surgery per day. Immediately after surgery, rats will be injected with an analgesic drug (probably: Flunixin). Rats will only be moved to their animal room at 1 hour after surgery. During this period, the wound, breathing and activity of the animal is checked every 15 minutes. During the at least 7-days-lasting recovery time, the condition of the animals will carefully be monitored (water consumption, weight, behavior, condition of the wound, condition of the fur) and rats will be treated once daily with an antibiotic drug: (probably: Cefazolin).

Normal and compulsive self-administration (exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b):

- 18 Consecutive days of short access (1h daily sessions) or long access (6 h daily sessions) to cocaine (presumable dose: 0.5 mg/kg/infusion) or methamphetamine (presumable dose: 0.05 mg/kg/infusion) self-administration. [redacted]

2 Days of Porsolt swim test (exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b):

- Daily placement of the rat into a glass cylinder filled with water. We will score immobile behavior during the first and second exposure to the water (day 1: 15 min, day 2: 5 min) in order to verify whether the intake of cocaine or methamphetamine has resulted in a 'depression-like state'. Alternative approaches do not work in rats (e.g. the tail suspension test for mice), require additional surgery (e.g. intracranial self-administration), interfere with the same brain systems as cocaine and methamphetamine do (e.g. the sucrose preference test), or reflect more 'anxiety-like', instead of 'depression-like', behavior (e.g. elevated plus-maze).

Extinction (exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b):

- Rats are again placed in the self-administration chambers (4 h per day for 10 days) while cocaine or methamphetamine is replaced by saline. Extinction is necessary to measure the below-mentioned re-instatement of psychostimulant taking behavior.

Re-instatement (exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b):

- Re-exposure to cocaine or methamphetamine self-administation for 5 consecutive days. Re-instatement after extinction is the most commonly used procedure to measure 'relapse' to drug addiction. The re-instatement of drug abuse after abstinence is a landmark feature of drug dependence.

Decapitation (exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b):

- 24 Hours after the self-administration experiments are finished, the brains of the animals are collected using a guillotine. These brains are used to measures the local changes in gene expression.

Total duration of the various experiments:

- exp 3a/b, 4a/b, 5a/b, 6a/b/c/d, 7a/b: [redacted]

Justification of approach:

- Self-administration is the most used technique to measure the intake of drugs of abuse in animals. It has high face validity to the human situation and is perfect to test the effects of anti-addiction treatments. Compared to repeated systemic injections of drugs of abuse, in the self-administration procedures the intake of these drugs in voluntary and less stressful (no injections). [redacted] The suggested procedures are based on literature and previous experience ([redacted] If minor changes have to be made, this will first be discussed with the institutional Welfare Officer (IVD). If necessary, an amendment will be submitted to the Central Authority for Scientific Procedures on Animals (CCD).

Note 1: Only the rats of experiment 5 are submitted to a double surgery procedure.
Note 2: The number of and time between the short or long access self-administration sessions as well as the order of the various tests are identical for all the animals ([redacted]'). To ensure that, at any given time, test and control rats are exposed to the same conditions, the animals of every single experiment will be equally distributed across test and control groups.

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

The minimum number of animals to be used in every single experiment is calculated using a power analysis (see section B1 for details). After reaching a milestone (see section 3.4.3: coherence), data will first be analyzed and follow-up studies will only be performed if the previous studies have revealed promising results. By using this 'go / no go' approach (see also section 3.4.3: coherence) we will significantly reduce the risk of performing animal studies that should not have been performed.

Data will be statistically analyzed using a repeated ANOVA [redacted] and self-administration sessions (exp 3a/b, 4a/b) [redacted] treatment and self-administration sessions (exp 5a/b, 6a/b/c/d, 7a/b).

B. The animals

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

The total maximum number of animals to be used in this animal procedure is 1792. The animal origin is decribed in the Table.

Justification of the animals:

We seek permission to perform our studies in [redacted] rats because [redacted] are the main proteins regulating respectively the re-uptake and intra-[redacted] levels of serotonin. Only male rats will be used because fluctuations in the levels of stress hormones, associated with the menstrual cycle of female rats, may negatively affect the outcome of the suggested studies. The rats have to be adult to ensure that the diameter of the jugular vein is maximal.

Treatment groups:

3a Self-administration in [redacted] rats:

[redacted] short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
constitutive [redacted] WT: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

3b Self-administration in constitutive [redacted] rats:

constitutive [redacted] short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
constitutive [redacted] WT: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

4a Self-administration in conditional [redacted] rats:

conditional [redacted] short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
conditional [redacted] WT: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

4b Self-administration in conditional [redacted] rats:

conditional [redacted] short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
conditional [redacted] WT: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

5a Self-administration in constitutive or conditional [redacted] rats with optogenetically reduced serotonin:

[redacted] control vector: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14
WT control vector: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14
[redacted] serotonin decreasing vector: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14
WT serotonin decreasing vector: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14

Remark: the group of rats to [redacted] is depending on the results of experiments 3 and 4. Because

of financial reasons, [redacted] rats are preferred above conditional [redacted] rats, unless the first group of rats does not display a change in psychostimulant self-administration.

5b Self-administration in [redacted] rats with optogenetically e[redacted] serotonin:

[redacted] + control vector: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14
WT + control vector: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14
[redacted] + serotonin [redacted]: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14
[redacted] WT + serotonin [redacted]: short access: cocaine n=14, methamphetamine n=14; long access: cocaine n=14, methamphetamine n=14

Remark: the group of rats to be used to optogenetically enhance extracellular serotonin is depending on the results of experiments 3 and 4. Because of financial reasons, constitutive [redacted] rats are preferred above conditional [redacted] rats, unless the first group of rats does not display a change in psychostimulant self-administration.

6a Self-administration in constitutive or conditional [redacted] rats with [redacted] at adulthood:

[redacted] + saline + pCPA (within rat design) short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
[redacted] WT + saline + pCPA (within rat design): short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

[redacted]n is depending on the results of experiments 3 and 4.
[redacted], unless the first group of rats does not display a change in psychostimulant self-administration.

6b Self-administration in constitutive or conditional [redacted] rats with [redacted] at adulthood

[redacted] + saline + FLUOX (within rat design): short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
[redacted] WT + saline + FLUOX (within rat design): short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

Remark: the group of rats to be used to [redacted] is depending on the results of experiments 3 and 4. Because of financial reasons, constitutive [redacted] rats are preferred above conditional [redacted] rats, unless the first group of rats does not display [redacted].

6c Self-administration in constitutive or conditional [redacted] rats with [redacted] during development

[redacted] + normal diet: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
WT + normal diet: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
[redacted] + [redacted]: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

[redacted] WT + tryptophan-free diet: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

Remark: the group of rats to be used to pharmacologically reduce extracellular serotonin is depending on the results of experiments 3 and 4. Because of financial reasons, [redacted] rats are preferred above conditional [redacted] rats, unless the first group of rats does not display a change in psychostimulant self administration.

6d Self-administration in constitutive or conditional [redacted] rats with pharmacologically enhanced serotonin during development

[redacted] + normal diet: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
WT + normal diet: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
[redacted] + 5-HTTP-enriched diet: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
WT + 5-HTTP-enriched diet: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

[redacted] is depending on the results of experiments 3 and 4. Because of financial reasons, [redacted] rats, unless the first group of rats does not display a change in self administration.

7a Self-administration in [redacted] rats after environmental enrichment:

[redacted] + normal cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
WT + normal cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
[redacted] + enriched cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
WT + enriched cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

Remark: the group of rats to be used to test the effects of [redacted] is depending on the results of experiments 3 and 4. Because of financial reasons, [redacted] rats are preferred above conditional [redacted] rats, unless the first group of rats does not display a change in psychostimulant self administration.

7b Self-administration in constitutive or conditional [redacted] rats after environmental enrichment:

[redacted] + normal cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
WT + normal cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
[redacted] + enriched cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12
WT + enriched cage: short access: cocaine n=12, methamphetamine n=12; long access: cocaine n=12, methamphetamine n=12

Remark: the group of rats to be used to test the effects [redacted] depending on the results of experiments 3 and 4. Because of financial reasons, [redacted] rats are preferred above conditional [redacted] rats, unless the first group of rats does not display a change in psychostimulant self administration.

Power analysis:

Previous studies using psychostimulant-treated knock-out animals have revealed a significant genotype effect when the effect-size is 50% (mean [redacted] is 150% of mean WT). Using this effect size, together with the obtained standard deviation and an alpha-value of 0.05 and a power of 0.80 revealed a minimum number of 10 rats per treatment group. Per treatment group 2 rats have been added to compensate for the animals that have to be excluded from our study because of IV catheter obstruction (exp 3, 4, 6 and 7). In case IV surgery is combined with stereotactic surgery, an additional 2 animals have been added to compensate for incorrect placement of the guide cannula that is used to inject the viral vectors into the DRN (exp 5). If the number of animals has to be adjusted due to unforeseen circumstances, this will first be discussed with the institutional Welfare Officer (IVD). If necessary, an amendment will be submitted to the Central Authority for Scientific Procedures on Animals (CCD).

Species	Origin	Maximum number of animals	Life stage
exp 3a: Wistar [redacted] and [redacted] WT	Own breeding program	96	adult
exp 3b: Wistar [redacted] and [redacted] WT	Own breeding program	96	adult
exp 4a: Wistar [redacted] and [redacted] WT	Generated by Sage labs	96	adult
exp 4b: Wistar [redacted] and [redacted] WT	Generated by Sage labs	96	adult
exp 5a: Wistar [redacted] and [redacted] WT	Own breeding program	224	adult
exp 5b: Wistar [redacted] and [redacted] WT	Own breeding program	224	adult
exp 6a: Wistar [redacted] and [redacted] WT	Own breeding program	96	adult
exp 6b: Wistar [redacted] and [redacted] WT	Own breeding program	96	adult
exp 6c: Wistar [redacted] and [redacted] WT	Own breeding program	192	adult
exp 6d: Wistar [redacted] and [redacted] WT	Own breeding program	192	adult
exp 7a: Wistar [redacted] and [redacted] WT	Own breeding program	192	adult
exp 7b: Wistar [redacted] and [redacted] WT	Own breeding program	192	adult

C. Re-use

Will the animals be re-used?

C. Re-use

[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:

To answer our research questions we need to study cocaine or methamphetamine self-administration in animals with a reduced expression of either [redacted] or [redacted] For this we use our unique [redacted] knock-out rats. Human subjects cannot be used because many people taking psychostimulants do also abuse other 'serotonin-changing' drugs like nicotine, alcohol and/or opiates. In addition, the amount of psychostimulant intake and the time between final drug use and the collection of brain tissue cannot be controlled in human subjects. Lower animals cannot be used because there is no self-administration equipment available (invertebrates) or the surgery procedures are relatively complicated (mice) leading to an unacceptable high exclusion of animals (i.e. mice in which the catheter could not be placed in the jugular vein). Cell-lines cannot be used because we need to measure behavior. From our previous studies we know that in rats self-administration is relatively easy to perform, resulting in reliable results.

Reduction:

We are using the absolute minimum number of animals necessary to still be able to discover potentially statistical significant differences between the various genotypes and/or treatments. Using even less animals leads to an increase of the standard error of mean (sem), thereby reducing the statistical power.

Refinement:

See section D2 below.

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

The discomfort our rats will be exposed to is limited to the absolute minimum necessary to answer our research questions. Surgery will be performed under isoflurane anesthesia. In addition, Lidocaine spray will be applied to the skin. Possible bleeding will be stopped using epinephrine pellets. In order to stay focused, small breaks are taken after the surgery of every 3 rats and no more than 12 rats will undergo surgery per day. After surgery, rats will be treated with Flunixin (analgesic drug), Cefazolin (antibiotic drug) and Heparin (anticoagulant). Rats will only be moved to their animal room at 1 hour after surgery. During this period, the wound, breathing and activity of the animal is checked every 15 minutes. During the recovery time of at least 1 week, the animal's condition will be monitored on a daily basis. All animals will be handled to familiarize them to the experimental procedures and cage enrichment, in the form of a small wooden block and nest material, will also be available.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

Accommodation and care

F. Accommodation and care

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

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

At least 2 weeks before IV surgery (exp 3, 4, 5, 6 and 7), rats will be housed in groups of 2 or 3, in an animal room with a reversed day/night cycle and shelters will be provided. After surgery, cagemates are removed because they will damage the cannula. Given that a collision with the sharp

edges of a shelter may result in the loss of the cannula, shelters are replaced by wooden blocks and nest material. Rats are housed under a reversed day/night cycle because the increased exploration during the testing period will significantly facilitate the self-administration training.

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.

I. Other aspects compromising the welfare of the animals

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

Due to stress after surgery, rats may be more afraid to human contact. However, after 3 days of handling, these rats show normal behavior again. The animal's welfare may also be affected by the Porsolt swim test, withdrawal from drug (ab)use, and a lack of a shelter or cagemates.

Explain why these effects may emerge.

Surgery, recovery from surgery, Porsolt swim test and the (temporary) removal of cocaine, methamphetamine, shelters and cagemates.

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

The discomfort our rats will be exposed to is limited to the absolute minimum necessary to answer our research questions (please note that drug withdrawal is necessary to test the re-instatement of drug taking behavior and removal of shelters and cagemates will significantly reduce the number of animals that have otherwise to be excluded from our study). Surgery will be performed under isoflurane anesthesia. In addition, Lidocaine spray will be applied to the skin. Possible bleeding will be stopped using epinephrine pellets. In order to stay focused, small breaks are taken after the surgery of every 3 rats and no more than 12 rats will undergo surgery per day. After surgery, rats will be treated with Flunixin (analgesic drug), Cefazolin (antibiotic drug) and Heparin (anticoagulant). Rats will only be moved to their animal room at 1 hour after surgery. During this period, the wound, breathing and activity of the animal is checked every 15 minutes. During the recovery time of at least 1 week, the animal's condition will be monitored on a daily basis. All animals will be handled to familiarize them to the experimenter and the experimental procedures and cage enrichment, in the form of a small wooden block and nest material, will also be available.

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.

If the animal looses its cannula or when the bodyweight after surgery stays below 85% of the pre-surgery weight for 3 consecutive days, animals will be euthanized using CO2. In case of unexpected changes in the animal's behavior (e.g. no self-grooming or no water intake), or when the animal does not respond to a stimulus anymore, it will also be sacrificed using CO2.

Indicate the likely incidence.

Previous studies have shown that less than 5% of the animals reach their humane endpoint.

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).

exp 3a/b, 4a/b, 6c/6d and 7a/b: moderate (one surgery + withdrawal from drug (ab)use), exp 5a/b and 6a/b: severe (two surgeries or one surgery + systemic injections + withdrawal from drug (ab)use).

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.

Rats will be sacrificed using a guillotine. Brains will be used to measure gene expression levels and, in case of experiment 5, to verify the location of viral vector injection.

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

> Retouradres Postbus 20401 2500 EK Den Haag

Radboud Universiteit Nijmegen
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6500 HB NIJMEGEN

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
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0900 28 000 28 (10 ct/min)

Onze referentie
Aanvraagnummer
AVD103002015228
Bijlagen
2

Datum 28-08-2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Geachte heer/mevrouw [redacted]
Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 25 augustus 2015.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD103002015228. 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. Zodra uw aanvraag compleet is, ontvangt u binnen veertig werkdagen een beslissing op uw aanvraag. 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 wordt uw aanvraag buiten behandeling 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.zbo-ccd.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	10300
Naam instelling of organisatie:	Radboud 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:	Postdoc
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	Onderzoeker in opleiding
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens verantwoordelijke uitvoering proces

Naam:	[redacted]
Functie:	Instantie voor Dierenwelzijn
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	instantievoordierenwelzijn@radboudumc.nl

Gegevens gemachtigde

BSN:	[redacted]
Naam:	[redacted]
Postbus:	9101
Postcode en plaats:	6500 HB NIJMEGEN
Wilt u een nieuwe machtiging afgeven?	Ja
Wat mag de gemachtigde doen?	[ ] Een projectvergunning aanvragen [ ] Een wijziging op een verleende projectvergunning aanvragen [ ] Een melding doorgeven op een verleende projectvergunning [ ] Een bezwaarschrift indienen en daarover communiceren met de Centrale Commissie Dierproeven en alle andere handelingen verrichten die nodig zijn voor een goede afwikkeling van het bezwaarschrift [ ] Alle bovenstaande opties

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:	25 september 2015
Geplande einddatum:	25 september 2020
Titel project:	[redacted]
Titel niet-technische samenvatting:	Nieuwe inzichten in en behandeling van cocaine en methamphetamine verslaving
Naam DEC:	RU DEC
Postadres DEC:	Postbus 9101, 6500 HB Nijmegen ([redacted]
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 741,-
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] Melding Machtiging [x] DEC-advies

Ondertekening

Naam:	[redacted]
Functie:	Instantie voor dierenwelzijn
Plaats:	Nijmegen
Datum:	25 augustus 2015

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Radboud Universiteit Nijmegen
p/a Miriam [redacted]
Postbus 9101
6500 HB NIJMEGEN

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

Onze referentie
Aanvraagnummer
AVD103002015228

Bijlagen
2

Datum 28-08-2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 28 augustus 2015
Vervaldatum: 27 september 2015
Factuurnummer: 201570228

Omschrijving	**Bedrag**
Betaling leges projectvegrunning dierproeven Betreft aanvraag AVD103002015228	€ 741,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL28RBOS 056.99.96.066 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 20401, 2500 EK te 's Gravenhage.

Radboud universitair medisch centrum
Concernstaf, sectie Kwaliteit en Veiligheid

Postbus 9101, 6500 HB Nijmegen
Huispost 628
Geert Grooteplein 10

Directeur
Mw. mr. W.M.J. Boeijen
www.radboudumc.nl

KvK 41055629/4

Geert Grooteplein 10
Postbus 9101
6500 HB Nijmegen

Datum
25 augustus 2015

Instantie voor Dierenwelzijn

Onderwerp
Factuurinformatie Projectaanvraag

Geachte CCD,

Hierbij sturen wij u de administratieve gegevens behorend bij de ingediende projectaanvraag. Wij verzoeken u de factuur te versturen naar de IvD als gemachtigde van de vergunninghouder. Hiervoor AUB het bij u bekend e-mailadres gebruiken (instantievoordierenwelzijn@radboudumc.nl).

Om verwerking door de financiële afdeling mogelijk te maken verzoeken wij u tevens ***op de factuur*** de volgende gegevens te vermelden:

Factuuradres:	Radboudumc 28 F&A crediteuren Postbus 9101 6500HB, Nijmegen
Kostenplaats en kostensoort:	040823-461220
CDL projectnummer:	2015-0021
Verantwoordelijk onderzoeker:	[redacted]

Bij voorbaat dank.

Met vriendelijke groeten

[redacted]
Instantie voor Dierenwelzijn
instantievoordierenwelzijn@radboudumc.nl

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen
[redacted]
Postbus 9101
6525EZ Nijmegen

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
www.centralecommissiedierproeven.nl

T 0900-2800028 (10 ct /min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD103002015228

Bijlagen
1

Datum 21 september 2015
Betreft Beslissing aanvraag projectvergunning dierproeven

Geachte mevrouw [redacted],

Op 27 augustus 2015 hebben wij uw aanvraagformulier voor een projectvergunning dierproeven ontvangen. Het gaat om uw project 'Psychostimulants: uptake or synthesis?' met aanvraagnummer AVD103002015228. Wij hebben uw aanvraag beoordeeld.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning. De onderzoeker zal zowel de go/no-go momenten als de criteria om een optionele behandeling uit te voeren met de IvD afstemmen. U kunt met uw project [redacted] starten. De vergunning wordt afgegeven van 25 september 2015 tot en met 25 september 2020.

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

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie RUDEC gevoegd. Dit advies is opgesteld op 24 augustus 2015. 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. Wij nemen dit advies van de commissie over, inclusief de daaraan ten grondslag liggende motivering.
Dit advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving liggen ten grondslag aan 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.zbo-ccd.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven
namens deze:

[redacted]

ir. G. de Peuter
Algemeen Secretaris

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

Bijlagen
- Vergunning
  - Hiervan deeluitmakend:
    - 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: Geert Grooteplein-Noord 9
Postcode en woonplaats: 6525EZ Nijmegen
Deelnemersnummer: 10300

deze projectvergunning voor het tijdvak 25 september 2015 tot en met 25 september 2020, voor het project [redacted]?' met aanvraagnummer AVD103002015228, volgens advies van Dierexperimentencommissie RUDEC.
De functie van de verantwoordelijk onderzoeker is Postdoc.
De aanvraag omvat de volgende bescheiden:

1. een aanvraagformulier projectvergunning dierproeven, ontvangen per post op 27 augustus 2015;
2. de bij het aanvraagformulier behorende bijlagen:
    a. Projectvoorstel, zoals ontvangen bij digitale indiening op 25 augustus 2015;
    b. Niet-Technische Samenvatting van het project, zoals ontvangen bij digitale indiening op 25 augustus 2015;
    c. Advies van dierexperimentencommissie RUDEC d.d. 24 augustus 2015 en ontvangen op 25 augustus 2015.

Dierproeven

Naam dierproef	**Diersoort**	**Aantal dieren voor het vergunde tijdvak**	**Ernst**
Experiments 1-2: microdialysis + [redacted] release	Ratten; Wistar; [redacted] en [redacted] en WT; volwassen	280	Licht en matig
Experiments 3-7: self-administration	Ratten; Wistar; [redacted] en [redacted] en WT; volwassen	1792	Licht, matig en ernstig

Beoordeling achteraf
Na afloop van dit project wordt een beoordeling achteraf uitgevoerd. Deze beoordeling zal uiterlijk 25 september 2021 plaatsvinden.

Voorwaarde:
Op grond van artikel 10a1 lid 2 Wet zijn aan een projectvergunning voorwaarden te stellen:

De onderzoeker zal zowel de go/no-go momenten als de criteria om een optionele behandeling uit te voeren met de IvD afstemmen.

In Artikel 10, eerste lid, onder a, Wet op de dierproeven, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van

deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in overleg met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarden wijzigen of intrekken.

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister van Economische Zaken 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 13c 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 13d 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. In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van een beoordeling achteraf.
Deze beoordeling zal uiterlijk 25 september 2021 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 van lijden van de proevendieren conform de vergunning waren.