Centrale Commissie Dierproeven

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

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

1 Gegevens aanvrager

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

☒ Ja > *Vul uw deelnemernummer in* 10300
☐ Nee > *U kunt geen aanvraag doen*

1.2 Heeft u al een AVD nummer?

☐ Ja > *Vul uw AVD nummer in* AVD1030020197744
☐ Nee

1.3 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag
Komt de aanvraag in aanmerking voor de vereenvoudigde procedure? ☐ Ja >Ga verder met vraag 1.4 ☐ Nee >Ga verder met vraag 1.4
☐ Wijziging of melding > Ga verder met vraag 2.1

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

Naam instelling of organisatie	Stichting Katholieke Universiteit Nijmegen			
Titel, voorletters en achternaam van de portefeuillehouder	Titel: Prof. dr.	Voorletters: [redacted]	Achternaam: [redacted]	☒ Dhr. ☐ Mw
E-mailadres contactpersoon				
Titel, voorletters en achternaam van de diens gemachtigde (indien van toepassing)	Titel	Voorletters: [redacted]	Achternaam: [redacted]	☐ Dhr. ☐ Mw
E-mailadres gemachtigde	instantievoordierenwelzijn@radboudumc.nl			

Vul de gegevens van het postadres in.

Straat en huisnummer	Geert Grooteplein		29 / HP231
Postcode en plaats	6525 EZ	Nijmegen	
Postbus, postcode en plaats	9101	6500 HB	Nijmegen

1.5 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

	E-mailadres	[redacted]@radboudumc.nl	
1.6 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.	(Titel) Naam en voorletters	[redacted]	☐ Dhr. ☒ Mw.
	Functie	[redacted]	
	Afdeling	[redacted]	
	Telefoonnummer		
	E-mailadres	[redacted]@radboudumc.nl	
1.7 *(Optioneel)* Vul hier de gegevens in van de persoon aan wie de portefeuillehouder de verantwoordelijkheid inzake de algemene uitvoering van het project en de overeenstemming daarvan met de projectvergunning heeft gedelegeerd.	(Titel) Naam en voorletters		☐ Dhr. ☐ Mw.
	Functie		
	Afdeling		
	Telefoonnummer		
	E-mailadres		
1.8 *(Optioneel)* Vul hier de gegevens in van de Instantie voor Dierenwelzijn	Telefoonnummer	[redacted]	
	E-mailadres	instantievoordierenwelzijn@radboudumc.nl	

1.9 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 Gaat uw aanvraag over een *wijziging* op een vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn?

☒ Nee > Ga verder met vraag 2.2

☐ Ja > Geef hier onder weer wat deze wijziging inhoudt en onderbouw deze. Antwoord dan in het projectplan en de niet-technische samenvatting de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier

2.2 Gaat uw aanvraag over een *melding* op een vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn?

☒ Nee > Ga verder met vraag 3

☐ Ja > Geef hier onder weer wat deze melding inhoudt en ga verder met vraag 6

3 Over uw project

3.1 Wat is de geplande start- en einddatum van het project?	Startdatum	01-06-2019
	Einddatum (t/m)	31-05-2023
3.2 Wat is de titel van het project?	Unveiling the mechanism(s) underlying the switch to mania during antidepressant treatment: The role of glutamate	
3.3 Wat is de titel van de niet-technische samenvatting?	De glutamaterge mechanismen die ten grondslag liggen aan mania geïnduceerd door antidepressiva	
3.4 Wat is de naam van de Dierexperimentencommissie	Naam DEC	RU DEC
	Postadres	Postbus 9101, 6500 HB Nijmegen (HP 231)

(DEC) van voorkeur?	E-mailadres	dierexperimentencommissie@radboudumc.nl

4 Factuurgegevens

4.1 (optioneel) Vul de gegevens van het factuuradres in.

Naam: [redacted]	Afdeling: [redacted]	
Straat: Geert Grooteplein		Huisnummer: 29 / HP231
Postcode: 6525 EZ	Plaats: Nijmegen	
Postbus: 9101	Postcode: 6500 HB	Plaats: Nijmegen
E-mail: instantievoordierenwelzijn@radboudumc.nl		

4.2 (optioneel) Vul hier het ordernummer van de instelling in.

Ordernummer: [redacted], CDL projectnummer: 2019-0005

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel Aantal bijlage(n) dierproeven 3

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☐

6 Ondertekening

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

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de portefeuillehouder namens de instellingsvergunninghouder of gemachtigde (zie 1.9). 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	IvD
Plaats	Nijmegen
Datum	05-04-2019
Handtekening	

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.	Unveiling the mechanism(s) underlying the switch to mania during antidepressant treatment: The role of glutamate

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.

Psychiatric disorders are serious disabling conditions with an enormous economic impact on societies and public health systems. Among them, bipolar disorder (BD) is extremely difficult to treat, with few medications available, whose utilization is endowed with several limitations such as significant side effects and partial efficacy. BD is a recurrent condition that is generally characterized by episodes of mania, hypomania, and depression. The impact of BD on patients can be devastating, with up to 15% of patients committing suicide (1). BD is seen in both men and women, but women experience more rapid cycling (2). The average age at onset is 25 years. The lifetime prevalence of BD type I, the type of BD associated with severe mood elevation, is around 0.6% in the general population. Because complex clinical presentations contribute to high levels of misdiagnosis of BD, mainly as major depressive disorder, prevalence numbers may be underestimates. The World Health Organization World Health Report on Mental Health acknowledged BD as the ninth leading cause of years of 'healthy' life lost due to premature mortality and disability (disability adjusted life-years) (3). In 2004, BD accounted for an estimated 0.9% of the total global burden of disease worldwide (4). The main objectives of BD management are stabilization of mood, to prevent acute episodes, unwanted hospital admissions and suicides, and improving quality of life. The treatment of BD is difficult because of the opposite nature of its symptoms (i.e. depression and mania). Treatment of both depression and mania in BD is complex, because a switch to mania may occur during antidepressant treatment, and antipsychotic treatments that reduce mania can cause rebound depressive episodes. As mania involves a severe clinical condition, pharmacotherapy is necessary to protect the patient and environment quickly. One well-known clinical condition involves a switch to mania during use of imipramine (5). Imipramine is used to treat depression. It belongs to a group of medicines known as tricyclic antidepressants (TCA). These medicines are thought to work by increasing the activity of a chemical called serotonin in the brain (6).
Focus on etiology provides an opening to advance the understanding of mania. The heritability index of BD is estimated at 0.85 (7), indicating that BD has a high genetic predisposition. A meta-analysis showed that BD is strongly associated with the low activity short (s) allelic variant of the serotonin transporter (5-HTT)-linked polymorphic region (5-HTTLPR s-allele) (8,9). Genetic 5-HTT inactivation reduces clearance of synaptic serotonin and increases extracellular serotonin levels (10), which possibly explains higher levels of serotonin's metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid of BD patients (11). Genetic association studies have also linked dopamine transporter (DAT) polymorphisms to BD (12). Furthermore, striatal DAT levels are reduced in unmedicated BD patients (13,14). Reduced DAT levels slow the clearance of synaptic dopamine

and increase extracellular dopamine levels, allowing for greater metabolism of dopamine to homovanillic acid (HVA) (11). This likely drives the elevated HVA levels seen in the cerebrospinal fluid of mania patients (15). Increased synaptic dopamine levels are also seen in amphetamine-induced euphoria, hypomania, and mania (16). Together these findings illustrate that BD is strongly associated with both 5-HTT and DAT down-regulation and thereby increased serotonin and dopamine signaling, not only genetically but also molecularly and neurochemically. Hence, 5-HTT and DAT effects may be more extensive than the small effect sizes typically associated with single genes. To increase the understanding of mania, animal models are of great importance, provided that they have translational value. Mania-like behavioural abnormalities, particularly hyperactivity, can be induced in rodents by interventions that alter the mesolimbic dopamine pathway. Two such approaches involve acute amphetamine administration (17) and genetic mutation of the Clock gene (18). While these models have face and predictive validity, they lack construct validity; there is no evidence for circadian gene mutations in the majority of BD patients. In line with the association between BD and the 5-HTTLPR s-allele, 5-HTT knockout (KO) rats, which exhibit increased extracellular serotonin but unaltered dopamine levels (19), demonstrate both depression-like (reduced sociability, anhedonia and behavioural despair (20,21) and mania-like (increased switching, slightly increased motivation and psychostimulantinduced hyperactivity (22,23,24) phenotypes. Interestingly, while 5-HTT KO rodents show normal activity at baseline, hyperactivity is seen in response to psychostimulants (23,25). In response to an atypical antipsychotic with antidepressant effects 5-HTT KO rats furthermore 'switch' from most to least conditioned freezing (26). DAT KO rodents show increased dopamine and HVA levels, and mania-like switching, hyper-motivation, hyperactivity and risky decision making (27, 28). 5-HTT and DAT KO animals thus overlap in switching (and motivation), while the other mania-like phenotypes in DAT KO animals likely require a monoaminergic challenge to emerge in 5-HTT KO animals. Hence, 5-HTT KO rats display a mixed depression- and mania-like profile with triggers needed for a shift to predominantly mania, and DAT KO rats constitutively display mania-like phenotypes.

One theory postulates that BD pathophysiology is due to glutamate hyperexcitability, which is thought to relate to energy metabolism via astrocytic functions (29). Magnetic resonance spectroscopy studies have revealed that BD patients show a consistent increase in glutamate levels in frontal brain areas and basal ganglia (including the striatum) compared to healthy controls (30,31), but the metabolic state during mania specifically is not clear. The glutamatergic hyperexcitability may be related to increased glutamate transmission, since the expression of AMPA and NMDA receptor subunits as well as the glial glutamate transporter were found to be decreased in the prefrontal cortex (32) and habenula. In the striatum of DAT KO rats, there are no changes in glutamate system components in the whole homogenate, but reduced expression in the post-synaptic density and increased expression at extrasynaptic sites, suggesting reduced synaptic retention and increased trafficking toward the extra-synapse. Specifically, the antiporter xCT is up-regulated, indicative for increased glutamate overflow at extrasynaptic sites, presumably leading to glutamate hyperexcitability. In DAT KO mice, synaptic plasticity studies also point to glutamatergic hyperexcitability in the prefrontal cortex (PFC) (33). Specifically, DAT KO mice exhibit a reduction in NMDA receptor number and/or function in the PFC, explained by a redistribution of NMDA receptors subunit from neuron surface to intracellular compartment under hyperdopaminergic conditions (33). Previously we found hyperexcitability of glutamatergic neurons in the cortex of 5-HTT KO rats as well (34).

The prefrontal cortex, striatum and lateral habenula (LHb), where we observed features of glutamatergic hyperexcitability in 5-HTT and DAT KO rats, play a key role in BD. Prefrontal glutamate signaling mediates top-down control over subcortical areas processing reward (e.g. striatum) and sociability/emotion (e.g. LHb) (35,36). Increased prefrontal glutamate levels (37), a significant deficit in cortical inhibition (38), and prefrontal cortical thinning (39) in BD patients are indicative for reduced top-down control. There is furthermore evidence for reduced top-down control in individuals with DAT and 5-HTT down-regulation (40,41,42). The striatum processes reward predictions through dopamine and glutamate neurotransmission (43), which is thought to promote hyperactivity as seen in mania (44,45). The PFC innervates the LHb (35), which functions as

anti-reward node; its glutamatergic activity increases when the individual experiences depressive mood, and decreases when the individual experiences reward (46). It has been suggested that dysregulation of the glutamatergic system in the LHb is implicated in the switch from depression to mania in BD (47). In sum, by considering all mentioned information, we hypothesize that mania is attributed to serotoninand dopamine-mediated glutamatergic hyperexcitability in the PFC-striatum-LHb circuitry, with both excessive dopamine and serotonin reducing prefrontal cortical top-down control, dopamine contributing to increased striatum-dependent reward processing and serotonin providing a permissive role for antidepressant-induced inhibition of LHb activity and depressive mood. Additionally, we aim to test how to pharmacologically rescue glutamatergic hyperexcitability, prevent a switch to mania-like behaviour under antidepressant imipramine treatment and reduce mania-like phenotypes in response to imipramine plus the human approved glutamate reducing drugs. One drug of interest is N-acetylcysteine, which, by interacting with the glutamate exchanger xCt, modulates the efflux of glutamate at extrasynaptic sites and stimulates the presynaptic receptor mGluR2 to inhibit glutamate release (48). Another option is imipramine plus LY2140023, an mGluR2 agonist, to inhibit glutamate release (49). Furthermore, imipramine plus memantine, an NMDA receptor antagonist effectively treating BD in clinical trials (50,51), may well prevent a switch to mania in 5-HTT KO rats and reduce mania-like phenotypes in DAT KO rats. These glutamatergic agents are generally well tolerated.

Glossary for terms used throughout the application:
Bipolar disorder (BD)
Tricyclic antidepressants (TCA)
Serotonin transporter (5-HTT)
5-hydroxyindoleacetic acid (5-HIAA) Dopamine transporter(DAT)
Homovanillic acid (HVA)
Knockout (KO)
Prefrontal cortex (PFC)
Lateral habenula (LHb)

References:

1. Medici CR et al. J Affect Disord 2015;183:39-44.**2.** Arnold LM. Psychiatr Clin North Am 2003;26:595-620. **3.** http://www.who.int/whr/2001/en/whr01_en.pdf. **4.** http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf. **5.** Tondo L et al. Acta Psychiatr Scand 2010;121:404-414. **6.**https://www.mayoclinic.org/drugs-supplements/imipramine-oral-route/description/drg 20072148. **7.** McGuffin P et al. Arch Gen Psychiatry 2003;60:497-502. **8.** Frye M et al. J Clin Psychiatry 2015;76:174-180. **9.** Jiang HY et al. Neurosci

Lett 2013;549:191-196. **10.** Homberg JR et al. Neuroscience 2007;146:1662-1676. **11.** Palsson E et al. J Neural Transm 2017; 24:11351143. **12.** Pinsonneault J et al. Neuropsychopharmacol 2011;36:1644-1655. **13.** Horschitz S et al. Mol Psychiatry 2005;10:1104-1109. **14.** Anand A et al. Bipolar Disord 2011;13:406-413. **15.** Gerner RH et al. Am J Psychiatry 1984;141:1533-1540. **16.** Mick E et al. Am J Med Genet B Neuropsychiatr Genet 2008;147B:1182-1185. **17.** Martinowich K et al. J Clin Invest 2009;119:726-736. **18** Mukherjee S et al. Biol Psychiatry 2010;68:503-511. **19.** Verheij MM et al. Eur Neuropsychopharmacol 2014;24:1850-1854. **20.** Olivier JD et al. Neuroscience 2008;152:573-584. **21.** Homberg JR et al. Psychopharmacology 2007;195:175-182. **22.** Nonkes LJ et al. Addict Biol 2013;18:434-440. **23.** Homberg JR et al. Psychopharmacology 2008;200:367-380. **24.** Nonkes LJ et al. Behav Brain Res 2014;259:268-273. **25.** Homberg J et al. BMC Genet 2010;11:37. **26** . Luoni A et al. Int J Neuropsychopharmacol 2013;16:1319-1330. **27.** Leo D et al. J Neurosci 2018;38:1959-1972. **28.** Milienne-Petiot M et al. Neuropharmacology 2017;113:260-270. **29.** Jun C et al. Exp Neurobiol 2014;23:28-35. **30.** Yildiz-Yesiloglu A. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:969-995. **31.** Kubo H et al. J Affect Disord. 2017;208:139-144. **32.** Karel P et al. Addict Biol 2016. **33.** Xu TX et al. J Neurosci 2009;29:14086-14099. **34.** Miceli S et al. *Cereb Cortex* 2017;1-17. **35.**Benekareddy M et al. Biol Psychiatry 2018;83:607-617. **36.** Ghazizadeh A et al. J Neurosci 2012;32:726-737. **37.** Yildiz-Yesiloglu A. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:969-995. **38.** Levinson AJ et al. J Clin Psychopharmacol 2007;27:493-497. **39.** Hanford LC et al. Bipolar Disord 2016;18:4-18. **40.** Homberg JR. Biol Psychiatry 2011;69:513-519. **41.** Bertolino A et al. J Neurosci 2006;26:3918-3922. **42.** Caldu X et al. Neuroimage 2007;37:1437-1444. **43.** Bamford NS et al. Neuron 2018;97:494510. **44.** Queiroz AI et al. Metab Brain Dis 2015;30:1207-1215. **45.** Bastos JR et al. J Psychiatr Res 2018;102:142-149. **46.** Batalla A et al. Neurosci Biobehav Rev 2017;80:276-285. **47.** Loonen AJM et al. Front Neural Circuits 2017;11:35. **48.** Baker DA et al. *J Neurosci* 2002;22:9134-9141. **49.** Li M et al. *Prog Neuropsychopharmacol Biol Psychiatry* 2015;60:66-76. **50.** Koukopoulos A et al. *Bipolar Disord* 2010;12:348-349. **51.** Koukopoulos A et al. *J Affect Disord* 2012;136:163-166.

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?

Bipolar Disorder (BD) is generally characterized by episodes of mania, hypomania, and depression. A well-known clinical feature of BD involves the switch from depression to mania during antidepressant treatment. The mechanisms underlying this switch are still elusive. Accordingly, our project will address the neurobiological mechanisms underlying the switch to mania during imipramine antidepressant treatment by taking advantage of the availability of two animal models, the serotonin transporter knockout (KO) rat and the dopamine transporter KO rat models. We hypothesize that the neurotransmitters serotonin and dopamine increase levels of the neurotransmitter glutamate in a brain circuitry that deals with mood states like

mania. Our study will provide a proof-of-principle of the glutamatergic mechanisms underlying mania by integrating behavioural, molecular, electrophysiological techniques.

Objectives:

- In the first experiment we would like to investigate whether the antidepressant imipramine reduces depression-like and induces mania-like behavior in 5-HTT knockout rats, and whether DAT knockout rats display mania-like phenotypes.
- In the second experiment we study the effect of imipramine treatment in 5-HTT and DAT knockout rats on the expression of glutamate system components at molecular level.
- In the third experiment we study the effect of imipramine treatment in 5-HTT and DAT knockout rats on the expression of glutamate system components at physiological level.
- In the fourth experiment we would like whether the antidepressant imipramine is associated with changes in glutamate neurotransmission in a brain circuit that deals with mood states in 5-HTT and DAT knockout rats.
- In the fifth experiment we would like to investigate whether a glutamatergic pharmacological agent prevents imipramine-induced manialike behavior.

Feasibility:

Our lab has the needed experience and facilities in-house to perform address these objectives. Our group has strong expertise in behavioural studies, in vivo microdialysis and electrophysiology, the techniques that will be applied in this project.

References:

Verheij MMM, Contet C, Karel P, Latour J, van der Doelen RHA, Geenen B, van Hulten JA, Meyer F, Kozicz T, George O, Koob GF, Homberg JR. Median and Dorsal Raphe Serotonergic Neurons Control Moderate Versus Compulsive Cocaine Intake. Biol Psychiatry. 2018 Jun 15;83(12):1024-1035. Karel P, Almacellas-Barbanoj A, Prijn J, Kaag AM, Reneman L, Verheij MMM, Homberg JR. Appetitive to aversive counter-conditioning as intervention to reduce reinstatement of reward-seeking behavior: the role of the serotonin transporter. Addict Biol. 2018 Jan 2. doi: 10.1111/adb.12596. Alvandi MS, Bourmpoula M, Homberg JR, Fathollahi Y. Association of contextual cues with morphine reward increases neural and synaptic plasticity in the ventral hippocampus of rats. Addict Biol. 2017 Nov;22(6):1883-1894.

Miceli S, Nadif Kasri N, Joosten J, Huang C, Kepser L, Proville R, Selten MM, van Eijs F, Azarfar A, Homberg JR, Celikel T, Schubert D. Reduced Inhibition within Layer IV of Sert Knockout Rat Barrel Cortex is Associated with Faster Sensory Integration. Cereb Cortex. 2017 Feb 1;27(2):933949. Verheij MM, Karel P, Cools AR, Homberg JR. Reduced cocaine-induced serotonin, but not dopamine and noradrenaline, release in rats with a genetic deletion of serotonin transporters. Eur Neuropsychopharmacol. 2014 Nov;24(11):1850-4.

Nonkes LJ, Maes JH, Homberg JR. Improved cognitive flexibility in serotonin transporter knockout rats is unchanged following chronic cocaine selfadministration. Addict Biol. 2013 May;18(3):434-40.

3.3 Relevance

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

The treatment of BD is difficult because of the opposite nature of its symptoms (i.e. depression and mania). Furthermore, the available treatments treat symptoms but not the core mechanisms in the brain that are malfunctioning, and can cause severe side effects. Hence, there is need for novel approaches that target the primary malfunctioning mechanisms underlying BD, specifically mania. A well-known clinical feature of BD involves the switch from depression to mania during antidepressant treatment. The mechanisms underlying this switch are still elusive. Accordingly, our project will address the neurobiological mechanisms underlying the switch to mania, taking advantage of the availability of two animal models, the serotonin transporter knockout (KO) rat and the dopamine transporter KO rat model. We hypothesize that the neurotransmitters serotonin and dopamine increase levels of the neurotransmitter glutamate in a brain circuitry that deals with mood states. In the end, our project will, by integrating behavioural, molecular, electrophysiological in rats, provide a proof-of-concept of the glutamatergic mechanisms underlying mania. Furthermore, by testing whether imipramine + glutamate lowering drug prevents the imipramine-induced mania we provide proof-of-principle for a novel treatment regime.

References

Olesen, J., A. Gustavsson, M. Svensson, H. U. Wittchen, B. Jonsson, C. s. group and C. European Brain (2012). "The economic cost of brain disorders in Europe." Eur J Neurol **19**(1): 155-162.

3.4 Research Strategy

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

To address the behavioural part of our research (DAP1), we need to show that antidepressant imipramine treatment reduces depression-like and induces mania-like behavior. To do this, adult male and female 5-HTT and DAT KO rats, and wild-type controls will be exposed to imipramine or regular drinking water as control for 4 weeks and later chronic imipramine treatment through their drinking water for a maximum of 6 weeks. We are required to use both males and females because gender differences in the phenomenology and course of the illness have been reported among male and female with bipolar disorder. Some studies with clinical samples report that women with bipolar disorder experience fewer manic episodes and more episodes of depression compared to men with bipolar disorder (Taylor, 1987). Women have reportedly higher rates of bipolar depression and type II bipolar disorder (depression with hypomania), a greater likelihood of having depression precede mania or hypomania, are more likely to be hospitalized for mania and have a rapid□cycling course somewhat more often than men (Viguera, 2001). Sex differences in the phenomenology and course of bipolar disorder points to potential sex differences in underlying mechanisms, which are unknown up to date. And such sex differences could in turn influence the efficacy of interventions. Indeed, some studies about differences in the treatment response of male and female with bipolar disorder suggest that there may be sex differences in response to mood stabilizers (Kawa, 2005). Hence, findings in one sex may not generalize to the other sex. In this project we will test both male and female rats. The 'speed' of the antidepressant-induced switch to mania can be assessed through the mania-related tests. It is possible that mania is seen in the first test (motivation test) in some animals, but not others, while all animals show mania-like phenotypes in the subsequent mania-like tests. Or, it is possible that within the motivation test there is a delay in an increase in motivation. It is also possible that the level of mania differs per sex or that this is task dependent. The experiments will have to point this out. Mania in bipolar disorder involves a severe clinical condition, pharmacotherapy is necessary to protect the patient and environment quickly.

Since one of the wellknown clinical condition involves a switch to mania during use of imipramine, we choose imipramine in our research (Tondo, 2010). Behavioural tests will be performed during imipramine treatment in order to investigate antidepressant potency.
Based on data collected in this project (see also DAP 2) and from consortium partners, we also study whether it is possible to pharmacologically rescue glutamatergic hyperexcitability, and prevent a switch to mania-like behaviour under antidepressant imipramine treatment and reduce mania like phenotypes. To address this question, we will treat animals with N-acetylcysteine (800 mg/kg/day), Memantine 30 mg/kg/day or LY2140023 (30 mg/kg) together with imipramine, via the drinking water.
Prior to the main experiment, a pilot study will be executed with surplus 5-HTT or DAT animals. This pilot is necessary 1) to select those behaviours most responsive to the imipramine treatment, 2) to enable us to measure how much imipramine -water the animals drink.
In the ex vivo part of our project (DAP2), we will study the effect of imipramine treatment in male and female 5-HTT and DAT knockout rats on the expression of glutamate system components at molecular level. To do this, we will treat animals with imipramine through the drinking water. After 3 weeks the rats will be decapitated without anesthesia to remove the brain. This fresh tissue will be subjected to a Western blot assay to analyse the expression of critical determinants of the glutamate synapse. We also evaluate the electrophysiological properties of glutamatergic synapses in the PFC, striatum and LHb, *ex vivo*. To do this, rats will get imipramine via drinking water for 3 weeks and after decapitation, the brains will be used for *ex vivo* electrophysiology. BD pathophysiology is due to glutamate hyperexcitability (Jun, 2014) and mania is attributed to serotonin- and dopaminemediated glutamatergic hyperexcitability in the PFC-striatum-LHb circuitry. To assess this hypothesis in DAP3, we measure dopamine, serotonin and glutamate neurotransmitter levels in these brain regions. Prior to the main experiment of DAP3, a pilot study will be executed with surplus 5-HTT animals. This pilot is necessary to find the proper coordinates of the striatum, LHb and mPFC in the brain and the targeting of two areas in one animals, for the microdialysis experiment.

References:

Taylor, Michael Alan, and Richard Abrams. "Gender differences in bipolar affective disorder." Journal of affective disorders 3.3 (1981): 261-271.
Viguera, Adele C., Ross J. Baldessarini, and Leonardo Tondo. "Response to lithium maintenance treatment in bipolar disorders: comparison of women and men." Bipolar Disorders3.5 (2001): 245-252.
Kawa, Izabela, et al. "Gender differences in bipolar disorder: age of onset, course, comorbidity, and symptom presentation." Bipolar disorders 7.2 (2005): 119-125.
Tondo, L., G. Vázquez, and R. J. Baldessarini. "Mania associated with antidepressant treatment: comprehensive meta□analyticreview*Acta Psychiatrica Scandinavica* 121.6 (2010): 404-414.
Jun, Chansoo, et al. "Disturbance of the glutamatergic system in mood disorders." *Experimental neurobiology* 23.1 (2014): 28-35.

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 0: pilot study

Prior to the main experiment, a pilot study will be executed with 5-HTT KO and wild-type male and females rats. animals. This pilot will allow us to select those behaviours most responsive to imipramine administration, and will inform us on potential confounds of repeated testing. We will also assess the amount of imipramine ingested by the animals via drinking water, by measuring how much imipramine -water the animals drink. The results will serve as a go/no go decision point for the main experiment. Based on the pilot, we will decide whether to continue with our main experiment as it was proposed or whether to adapt the procedures (selection of behavioural tests) (Experiment 0A). The pilot study will also be used to practice stereotactic surgeries and to determine relevant coordinates for the micro dialysis experiments (Experiment 0B).

Experiment 1: Effect of antidepressant treatment on depression- and mania-like behaviour
In this experiment we study whether imipramine causes a switch from depression to mania in male and female SERT, but not DAT, knockout rats. The best way to test this is by having a within subject design, allowing us to test how imipramine within the same individual causes such a switch. To this end we first test depression- and mania-like behaviour under baseline conditions, and subsequently in response to imipramine treatment.
Treatment: To study the switch to mania during antidepressant treatment, male and female 5-HTT and DAT KO rats, and wild-type controls rats first are 'treated' with vehicle (water) for 4 weeks, and the behavioural test battery as set out below is conducted. Then, the animals are treated with imipramine (Sigma Aldrich). Imipramine (10 mg/kg/day) will be administered via drinking water for 6 weeks.
During treatment rats are again subjected to the behavioural tests. The treatment lasts for 10 weeks (or less, if the number of behavioural tests will be reduced based on the pilot study), to ensure that the animals are under imipramine treatment during behavioural testing.

***Behavioural testing*:**
Depression- and mania-like behavior are measured during vehicle and imipramine treatment. The animals will be exposed to all these tests twice, before and during treatment.
Since depression and mania consist of a constellation of phenotypes, we apply a test battery. Hence, a major strength of our approach is that conclusions will not rely on just one test for depression and one for mania. While 5-HTT KO rats show normal baseline activity, DAT KO rats show hyperactivity, which can be a confounding factor in behavioral tests. We control for activity by having non-programmed options in operant tasks, correcting scores for total responses, and excluding the forced swim test in data interpretation for DAT KO rats.

Sleep/wake pattern

The animals are placed in a Phenotyper (automated homecage of Noldus Information Technology), and are allowed to habituate to this new environment for a day. During the second and third day, their sleep wake rhythm will be monitored continuously.

Social preference

Rat are tested in three-chamber social test. During trial 1 (5 min) rats choose between an empty and social partner containing site of the test box. During Trial 2 (5 min) the rats choose between a familiar and a novel social partner (placed at opposite sites in the box).

Anhedonia/Motivation task

Rats will be tested in operant cages and trained 5/day during 1 hour sessions to lever press for sucrose pellets under a fixed ratio 1 schedule. Once rats have acquired sucrose self-administration, defined as at least 25 reinforced responses over 1-hr with less than ± 20% variation in responding from day-to-day, the rats will be subjected to a progressive ratio (PR) schedule to measure the motivation to self-administer sucrose. The PR schedule is based on the following equation: responses/injection [5 x e(injection number x 0.2)]-58. PR responding is assessed during one 3-hr sessions (Karel et al. submitted).

Puzzle box

The rats are placed in a so-called puzzle box in which rats have to solve obstacles to get from a lit place to a dark hidden place. The tests last for 3 days (3 trials per day, each trial lasting 4 minutes at max.). On day one rats can enter the dark hidden box freely (trial 1), and then have to use an underpass (trial 2 and 3). On day two trial 4 is similar to trials 2 and 3 from the previous day. During trials 5 and 6 on day two the underpass is filled with sawdust, which the rats have to remove to enter the dark hidden box. On day three, trial 7 is a repetition of trials 5 and 6 of the previous day). During trials 8 and 9 the rats have to move away cardboard that blocks the underpass with their teeth and paws (Ben Abdallah et al., 2011).

Psychostimulant-induced hyperactivity (open field test)
At week 4, rats will be placed in a novel arena (open field), which they are allowed to explore for 60 minutes. Subsequently they receive an intraperitoneal injection of saline and activity is measured for another 30 min. Finally, they receive an intraperitoneal injection of amphetamine and locomotor activity is assessed for 60 min. Behaviour is videotaped, and through tracking software we will analyze distance moved and time spent in center as a measure of anxiety.

Elevated plus maze test

The rats are placed in a plus-shaped arena which has two closed arms and two open arms. Their behaviour is videotaped over a trial of 5 minutes, during which the time spent and distance crossed in the open arms is used as a parameter for anxiety (Pellow et al., 1985).

Basal and stress-induced plasma corticosterone levels
Blood is collected through a tail cut 5 min prior to 30 min restraint stress, and 20 and 40 min after restraint stress. Plasma corticosterone levels are measured in the plasma ex vivo.

Forced swim test
The forced swim test is a well-characterized behavioural paradigm to define the level of depressive-related behaviour. The test relies upon the animal's tendency for survival, therefore to keep swimming; depressed animals will start floating earlier and be immobile for a longer time. The forced swim test exists of two parts: the induction phase and test phase. On the induction day rats are placed in a cylinder filled with water for 15 min, on the test day rats are placed in the cylinder for 5 min (Olivier et al., 2008). Time spent on swimming and floating (=psychomotor retardation) is measured.

***Sacrifice*:** 24 hours after the last behavioural test the rats on pharmacological treatment will be sacrificed by CO2.

Experiment 2: Effect of antidepressant treatment on gene expression levels of glutamate system components

Male and female 5-HTT and DAT KO rats and their respective controls will be treated for 3 weeks and sacrificed for molecular analyses. This group will not be subjected to behaviour tests and microdialysis in order to avoid effects on the testing and surgery on gene expression. Because the rats are not behaviourally tested and subjected to microdialysis, treatment will last 3 weeks, which is sufficient for chronic treatment in rodents (Lee, 2010). Furthermore, we apply a cross-sectional design, because rats have to be sacrificed for ex-vivo molecular measurements (a longitudinal design as in experiment 1 and 4 is thus not possible)

***Treatment*:** Rats will be treated with imipramine/vehicle for 3 weeks, and 24 hours after the last treatment day rats will be decapitated without anesthesia to avoids problems with gene expression changes. Brain will be rapidly removed from the skull, frozen on dry ice, and stored at -80 0C, until use for molecular analyses. After sacrificing the animals, we will investigate by Western blot analyses how serotonin and dopamine modulate physiologic glutamate homeostasis in the PFC, striatum and LHb.

Experiment 3: Ex vivo electrophysiology to elucidate the physiological characteristics of glutamatergic synapses due to antidepressant treatment

Male and female 5-HTT and DAT KO rats and their respective controls will be treated for 3 weeks and sacrificed for ex vivo electrophysiological recordings

***Treatment*:** Rats will get imipramine (10 mg/kg/day) or vehicle for 3 weeks (emerging mania (Lee, H. J, 2010)). After three weeks, animals will be decapitated without anesthesia to prevent anesthesia effect on electrophysiological features of the brain (Rammes, 2009) and also keep the conditions comparable between experiments 2 and 3. Then the brains will be removed to continue with the electrophysiological study.

Electrophysiology recording:

To further test the overall hypothesis, we will perform whole cell patch-clamp experiments in acute slices of the LHb to better characterize pre- and postsynaptic glutamatergic transmission. Dissection and slicing will be performed as for extracellular recording. For recording, slices will be transferred in a submersion recording chamber perfused with warm and oxygenated aCSF (28°C, 2 ml/min) and mounted on an upright microscope equipped for infrared visualization and patch-clamp recording. For recordings of evoked transmission (eEPSC), QX314 (5□mM) will be added to the internal solution and stria medullaris will be stimulated using twisted nickel-chrome electrodes. Presynaptic release probability will be assessed by paired-pulses stimulations (10 to 50 ms apart). Finally, NMDAR EPSCs will be recorded by stimulating the input stria medullaris fibre in a modified extracellular aCSF solution with the AMPAR blocker NBQX and 0 Mg2+.

References:

Lee, H. J., et al. "Chronic imipramine but not bupropion increases arachidonic acid signaling in rat brain: is this related to 'switching'in bipolar disorder?." *Molecular psychiatry* 15.6 (2010): 602.

Rammes, Gerhard, et al. "Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B subunit expression." *Neuropharmacology* 56.3 (2009): 626-636.

Experiment 4 (DAP3): Effect of antidepressant treatment on neurotransmitters level

To study the neurotransmitter changes during the antidepressant-induced switch to mania we will conduct a stereotactic surgery to place guide cannulas in the striatum, LHb and/or mPFC, treat the rats with vehicle for 1 week, measure neurotransmitter levels by in vivo microdialysis, subject the rats to imipramine for 3 weeks, and repeat the microdialysis experiment. Through this longitudinal design we will be able to assess changes in neurotransmitter levels in the same animals.

Treatment: *M*ale and female 5-HTT and DAT KO rats, and wild-type controls, will be treated with vehicle (water), and subsequently imipramine. Imipramine (10 mg/kg/day) will be administered via drinking water for 2 weeks, starting from week five to eight. Rats will undergo a surgery to unilaterally place guide cannulas into the striatum and LHb in the same animal. In other groups of animals, we will measure neurotransmitters in the medial PFC (mPFC) and LHb by unilaterally implanting two guide cannulas in the regions mentioned. The rats thus get two cannulas in their brain. This 1. saves animals, 2. does not cause more suffering for animals since it will be done during one surgery, and 3. Allows investigation of correlations between neurotransmission changes in the two areas. After recovery, animals will be treated with water (vehicle) for one week and will be subjected to an in vivo microdialysis study in which we assess dopamine, serotonin and glutamate levels in vivo in the LHb, mPFC and nucleus accumbens. Subsequently, the rats will be treated with imipramine for 3 weeks and the *in vivo* microdialysis experiment will be repeated. We measure off-line levels of i) dopamine and serotonin (as well as their metabolites), and ii) glutamate (as well as GABA and glutamine). The microdialysis probe is inserted into guide cannulas in the evening before the test day. On the test day baseline levels of neurotransmitter are measured during 3-4 hours (15 min samples). After the last microdialysis experiment, rats will be sacrificed by perfusion under deep anesthesia with pentobarbital (i.p), to obtain fixed brains for validation of the placement of the cannulas site.

Experiment 5 (DAP1): Pharmacological prevention of antidepressant-induced mania and aberrant glutamate neurotransmission

To determine whether there is a causal relationship between glutamatergic changes in the prefrontal cortex-striatum-LHb circuitry and the switch to mania during antidepressant treatment, we will test whether an FDA approved drug restoring glutamatergic hyperexcitability, as adjunctive to imipramine treatment, can reduce depression and mania-like phenotypes in 5-HTT and DAT knockout rats. This will be done as a first step for a next innovative intervention to prevent the relevant problem of mania switching induced by antidepressants. One drug of interest is N-acetylcysteine, which, by interacting with the glutamate exchanger xCt, modulates the efflux of glutamate at extrasynaptic sites and stimulates the presynaptic receptor mGluR2 to inhibit glutamate release. Another option is imipramine plus LY2140023, an mGluR2 agonist, to inhibit glutamate release. Furthermore, imipramine plus memantine, an NMDA receptor antagonist effectively treating BD in clinical trials, may well prevent a switch to mania in 5-HTT KO rats and reduce mania-like phenotypes in DAT KO rats.

Treatment: To study the switch to mania during antidepressant treatment, male and female 5-HTT and DAT KO rats, and wild-type controls rats are first are 'treated' with vehicle (water) for 4 weeks, and the behavioural test battery as set out in experiment 1 is conducted. Then, the animals are treated with imipramine ((10 mg/kg/day) via drinking water for 6 weeks. In combination with imipramine, animals will receive N-acetylcysteine (800

mg/kg/day) (Otte; 2011, Gürer; 1998), Memantine 30 mg/kg/day (Ihalainen; 2011, Gao; 2011) and LY2140023 (30 mg/kg) (Lowe; 2012) in the drinking water for 6 weeks.

***Behavioural testing*:**
Depression- and mania-like behavior will be measured during imipramine + N-acetylcysteine, imipramine + LY2140023 or imipramine + memantine treatment, starting 2 weeks after start of the treatment.

***Sacrifice*:** 24 hours after the last behavioural test the rats on pharmacological treatment will be sacrificed by CO2.

References:

Otte, David-Marian, et al. "N-acetyl cysteine treatment rescues cognitive deficits induced by mitochondrial dysfunction in G72/G30 transgenic mice." *Neuropsychopharmacology* 36.11 (2011): 2233.
Gürer, Hande, et al. "Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats." *Toxicology* 128.3 (1998): 181189.
Ihalainen, Jouni, et al. "Effects of memantine and donepezil on cortical and hippocampal acetylcholine levels and object recognition memory in rats." *Neuropharmacology* 61.5-6 (2011): 891-899.
Gao, Y., Payne, R. S., Schurr, A., Hougland, T., Lord, J., Herman, L., ... & El-Mallakh, R. S. (2011). Memantine reduces mania-like symptoms in animal models. *Psychiatry research*, *188*(3), 366-371.
Lowe, Stephen, et al. "Effects of a novel mGlu 2/3 receptor agonist prodrug, LY2140023 monohydrate, on central monoamine turnover as determined in human and rat cerebrospinal fluid." *Psychopharmacology* 219.4 (2012): 959-970.

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

Our project has 5 milestones, namely: pilot of DAP1, main experiments of DAP1 (experiment 1 and 5), main experiments of DAP2 (experiment 2 and 3), pilot of DAP3 and main experiment of DAP3 (experiment 4). Across experiments 5-HTT and DAT knockout rats are used. Upon finishing the pilot of DAP1, we will reach the first decision point before executing the actual experiment described in DAP1. Firstly, this pilot will show us the optimal behavioural tasks to assess depression and mania like behaviour induced by imipramine, and whether these tests can be repeatedly administered without major repetition effects. We expect that imipramine will significantly increase mania-like symptoms and decrease depression-like symptoms in 5-HTT KO rats on at least 2 tests. We also expect validation of mania-like symptoms in DAT KO rats in at least 2 tests (Demontis; 2015, Goldberg; 2003, Frye, 2009). In case we do not find effects of imipramine on behavioural tasks, we will discuss our next course of action during an interim consultation with AWB. Furthermore, the pilot will inform us on the efficacy of our imipramine administration route. If imipramine given through the drinking water is ineffective in inducing mania, we will ask for experimental adjustments through an amendment. For instance, another option would be to administer imipramine via oral gavage (drawback: more stress). The results from pilot 0A of DAP1 will use as go/no go for experiment 1 and 5

(DAP 1). In experiment 1, we study the effect of imipramine treatment on behaviour in relation to depression and mania like disorders. In DAP2 we will address how imipramine treatment affects expression levels of glutamate system components (experiment 2) or the electrophysiological features of neurons in different brain regions (experiment 3). DAP3 allows us to establish the effect of imipramine treatment on brain neurotransmitter level in relation to depression and mania like disorders. Prior to the main experiment, a pilot study (experiment 0B) will be executed with surplus animals to practice surgeries on these rats. Upon finishing pilot of DAP3, we can find the proper coordinates of the striatum, LHb and mPFC in the brain for the microdialysis experiment. In experiment 4 we measure the level of dopamine, serotonin (as well as their metabolites), and ii) glutamate (as well as GABA and glutamine) in different brain areas. Based on the molecular, electrophysiological and neurochemical data together, as well as data collected by other consortium members of other countries, we will decide about the best glutamatergic intervention. If there is no change in any of the glutamatergic measurements we will not conduct experiment 5. Hence, experiment 2, 3 and 4 serve as go/no go for experiment 5. In experiment 5, we will investigate the effect of imipramine plus N-acetylcysteine, LY2140023 or memantine (glutamatergic intervention) on depression- and mania-like behaviour.

References:

Demontis, Francesca, et al. "Memantine prevents "bipolar-like" behavior induced by chronic treatment with imipramine in rats." *European journal of pharmacology 752 (2015): 49-54.*

Goldberg, Joseph F., and Christine J. Truman. "Antidepressant inducedmania:an overview of current controversies." *Bipolar Disorders* 5.6 (2003): 407-420.

Frye, Mark A., et al. "Correlates of treatment-emergent mania associated with antidepressant treatment in bipolar depression." *American Journal of Psychiatry 166.2 (2009): 164-172.*

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	Behaviour
2	Ex vivo measurements
3	Surgery

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 Behaviour

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.

For the pilot study (0A), male and female 5-HTT KO and wild type rats will be treated with vehicle (water) for 4 weeks and later the same animals will be treated with imipramine in the drinking water for 6 weeks. They will be subjected to the behavioural tests in table 1 below, which also indicates the outcome parameters. These parameters are indicative for either depression-like or mania-like behaviour. This pilot will allow us to select those behaviours (**Table 1**) most responsive to imipramine administration, and will inform us on potential confounds of repeated testing. We expect significant increase in mania-like symptoms in 5-HTT KO rats after imipramine treatment on at least 2 tests, and significant decrease in depression-like symptoms in 5-HTT KO rats after imipramine treatment on at least 2 tests. We also expect validation of mania-like symptoms in DAT KO rats in at least 2 tests (Demontis; 2015, Goldberg; 2003, Frye, 2009). We will also assess the amount of imipramine ingested by the animals via drinking water, by measuring how much imipramine -water the animals drink. Based on the pilot study, we will decide which behavioural tests we include in experiment 1 and experiment 4.

In experiment 1, male and female 5-HTT and DAT KO rats and their wild-type controls will be treated with vehicle (water) for 4 weeks and later the same animals will be treated with imipramine in the drinking water for 6 weeks. During the first 4 weeks and also from week 7-10, they will be subjected to a selection of behavioural tests in table 1 below, in which we also indicate the primary (and secondary) outcomes. In this table we mention all tests, because we do not know upfront the outcome of the pilot study. The number of tests in experiment 1 thus is expected to be less than presented in the table (also reducing the treatment duration accordingly). After the behavioural study, rats will be sacrificed after the procedure by CO2.

In experiment 5, male and female 5-HTT and DAT KO rats and their wild-type controls will be treated vehicle (water) for 4 weeks (or less, if we reduce the number of behavioural tests based on the pilot study), and the behavioural test battery as set out below (**Table 1**) is conducted (as baseline). Later they will be treated with imipramine + memantine, Nacetylcysteine or LY2140023 in the drinking water (control: one of these drugs), for 6 weeks and again subjected to behavioural test battery (based on the outcome of the pilot study).

References:

Demontis, Francesca, et al. "Memantine prevents "bipolar-like" behavior induced by chronic treatment with imipramine in rats." *European journal of pharmacology* 752 (2015): 49-54.

Goldberg, Joseph F., and Christine J. Truman. "Antidepressant□inducedmania:an overview of current controversies." *Bipolar Disorders* 5.6 (2003): 407-420.

Frye, Mark A., et al. "Correlates of treatment-emergent mania associated with antidepressant treatment in bipolar depression." *American Journal of Psychiatry* 166.2 (2009): 164-172.

Green = depression-related test; Grey = mania-related test. Rats are tested in order of increasing stress.				
Type of measure	**Timing**	**Short test description**	**Primary outcome**	**Secondary outcome**
Sleep/wake pattern	Week 1 (3 days)	Rats are housed in Phenotyper cages and sleep/wake patterns are assessed	Activity during the day and night	Stereotypies, eating behaviour
Social preference	Week 1 (2 days)	Rat are tested in three-chamber social test. During trial 1 rats choice between an empty and social partner containing site of the test box. During Trial 2 the rats choose between a familiar and a novel social partner (placed at opposite sites in the box)	Time (s) spent on exploration of the social versus non-social sites of the box.	
Anhedonia/Motivation	Week 2 (5 days)	Train rats to obtain sucrose pellets in touchscreen boxes; fixed ratio 1 and progressive ratio	Number of responses	Break point
		BREAK		
Puzzle box	Week 3 (4 days)	Test rats in puzzle box in which rats have to solve obstacles to get from a lit place to a dark hidden place	Latency to get to dark hidden place	
		BREAK		
Psychostimulant-induced hyperactivity	Week 3 (1 day)	Place rat in open field box for habituation (1 hr), give saline (30 min), give amphetamine injection (2 hr) (see also WP2, 3 and 5)	Distance travelled (cm) over 10 min bins	-
		BREAK		
Elevated plus maze test	Week 4 (1 day)	Test rats explore elevated plus maze for 5 min	Open arm time	-
		BREAK		
Basal and stress-induced plasma corticosterone levels	Week 4 (1 day)	Blood is sampled through a tail cut 5 min before, and 15, 30 and 60 min after restraint stress	Plasma corticosterone levels	
		BREAK		
Behavioural despair	Week 4 (2 days)	Forced swim: Place rats in cylinder with water. Day 1: 5 min; day 2: 5 min	Time spent on (im)mobility	-

IMIPRAMINE TREATMENT for 2 weeks before behavioural tests starts IMIPRAMINE TREATMENT continues during the tests below Green = depression-related test; Grey = mania-related test. Rats are tested in order of increasing stress.				
Type of measure	**Timing**	**Short test description**	**Primary outcome**	**Secondary outcome**
Sleep/wake pattern	Week 7 (3 days)	Rats are housed in Phenotyper cages and sleep/wake patterns are assessed	Activity during the day and night	Stereotypies, eating behaviour
Social preference	Week 7 (2 days)	Rat are tested in three-chamber social test. During trial 1 rats choice between an empty and social partner containing site of the test box. During Trial 2 the rats choose between a familiar and a novel social partner (placed at opposite sites in the box)	Time (s) spent on exploration of the social versus non-social sites of the box.	
Anhedonia/Motivation	Week 8 (5 days)	Train rats to obtain sucrose pellets in touchscreen boxes; fixed ratio 1 and progressive ratio	Number of responses	Break point
BREAK				
Puzzle box	Week 9 (4 days)	Test rats in puzzle box in which rats have to solve obstacles to get from a lit place to a dark hidden place	Latency to get to dark hidden place	
BREAK				
Psychostimulant-induced hyperactivity	Week 9 (1 day)	Place rat in open field box for habituation (1 hr), give saline (30 min), give amphetamine injection (2 hr) (see also WP2, 3 and 5)	Distance travelled (cm) over 10 min bins	-
BREAK				
Elevated plus maze test	Week 10 (1 day)	Test rats explore elevated plus maze for 5 min	Open arm time	-
BREAK				
Basal and stress-induced plasma corticosterone levels	Week 10 (1 day)	Blood is sampled through a tail cut 5 min before, and 15, 30 and 60 min after restraint stress	Plasma corticosterone levels	
BREAK				
Behavioural despair	Week 10 (2 days)	Forced swim: Place rats in cylinder with water. Day 1: 5 min; day 2: 5 min	Time spent on (im)mobility	-

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

The duration of behavioural testing and experimental timeline is provided in Table 1.

Experiment 0: pilot study
Prior to the main experiment, a pilot study (experiment 0A) will be executed with 5-HTT KO and wild-type animals. This pilot will allow us to select those behaviours (Table 1) most responsive to imipramine administration, and will inform us on potential confounds of repeated testing. We will also assess the amount of imipramine ingested by the animals via drinking water, by measuring how much imipramine -water the animals drink.

Experiment 1: Effect of antidepressant treatment on depression- and mania-like behaviour
In this experiment we study whether imipramine causes a switch from depression to mania in male and female SERT, but not DAT, knockout rats (see pp for rationale). The best way to test this is by having a within subject design, allowing us to test how imipramine within the same individual causes such a switch. To this end we first test depression- and mania-like behaviour under baseline conditions, and subsequently in response to imipramine treatment.
Treatment: To study the switch to mania during antidepressant treatment, male and female 5-HTT and DAT KO rats, and wild-type controls rats are first are 'treated' with vehicle (water) for 4 weeks, and the behavioural test battery as set out below is conducted. Then, the animals are treated with imipramine (Sigma Aldrich). Imipramine (10 mg/kg/day) will be administered via drinking water for 6 weeks, starting from the week 5 to 10 until the day of sacrifice. Imipramine will dissolve in the drinking water at concentrations of 180mg/L (Broitman and et al.). This administration method was chosen due to its low stress factor, to ensure it does not confound our measurements. The efficacy of this administration method will be investigated in the pilot study during which intake will be measured every 2 days by monitoring imipramine-water consumption and bodyweight. Since the rats are socially housed, we take the average intake and average body weight as crude measure. Otherwise we would have to socially isolate the animals. However, we believe that stress of single housing does not weight against measures of water intake of each individual rat.
During treatment rats are again subjected to the behavioural tests below. The treatment lasts for 6 weeks (or less, if the number of behavioural tests will be reduced based on the pilot study), to ensure that the animals are under imipramine treatment during behavioural testing.

***Behavioural testing*:**
Depression- and mania-like behavior are measured during vehicle and imipramine treatment (**Table 1**). The animals will be exposed to all these tests twice, before and during treatment.
Since depression and mania consist of a constellation of phenotypes, we apply a test battery. Hence, a major strength of our approach is that conclusions will not rely on just one test for depression and one for mania. While 5-HTT KO rats show normal baseline activity, DAT KO rats show hyperactivity, which can be a confounding factor in behavioral tests. We control for activity by having non-programmed options in operant tasks, correcting scores for total responses, and excluding the forced swim test in data interpretation for DAT KO rats.

Sleep/wake pattern

The animals are placed in a Phenotyper (automated homecage of Noldus Information Technology), and are allowed to habituate to this new environment for a day. During the second and third day, their sleep wake rhythm will be monitored continuously.

Social preference

Rat are tested in three-chamber social test. During trial 1 (5 min) rats choose between an empty and social partner containing site of the test box. During Trial 2 (5 min) the rats choose between a familiar and a novel social partner (placed at opposite sites in the box).

Anhedonia/Motivation task

Rats will be tested in operant cages and trained 5/day during 1 hour sessions to lever press for sucrose pellets under a fixed ratio 1 schedule. Once rats have acquired sucrose self-administration, defined as at least 25 reinforced responses over 1-hr with less than ± 20% variation in responding from day-to-day, the rats will be subjected to a progressive ratio (PR) schedule to measure the motivation to self-administer sucrose. The PR schedule is based on the following equation: responses/injection [5 x e(injection number x 0.2)]-58. PR responding is assessed during one 3-hr sessions (Karel et al. submitted).

Puzzle box

The rats are placed in a so-called puzzle box in which rats have to solve obstacles to get from a lit place to a dark hidden place. The tests last for 3 days (3 trials per day, each trial lasting 4 minutes at max.). On day one rats can enter the dark hidden box freely (trial 1), and then have to use an underpass (trial 2 and 3). On day two trial 4 is similar to trials 2 and 3 from the previous day. During trials 5 and 6 on day two the underpass is filled with sawdust, which the rats have to remove to enter the dark hidden box. On day three, trial 7 is a repetition of trials 5 and 6 of the previous day). During trials 8 and 9 the rats have to move away cardboard that blocks the underpass with their teeth and paws (Ben Abdallah et al., 2011).

Psychostimulant-induced hyperactivity (open field test)

At week 4, rats will be placed in a novel arena (open field), which they are allowed to explore for 60 minutes. Subsequently they receive an intraperitoneal injection of saline and activity is measured for another 30 min. Finally, they receive an intraperitoneal injection of amphetamine and locomotor activity is assessed for 60 min. Behaviour is videotaped, and through tracking software we will analyze distance moved and time spent in center as a measure of anxiety.

Elevated plus maze test

The rats are placed in a plus-shaped arena which has two closed arms and two open arms. Their behaviour is videotaped over a trial of 5 minutes, during which the time spent and distance crossed in the open arms is used as a parameter for anxiety (Pellow et al., 1985).

Basal and stress-induced plasma corticosterone levels

Blood is collected through a tail cut 5 min prior to 30 min restraint stress, and 20 and 40 min after restraint stress. Plasma corticosterone levels are measured in the plasma ex vivo.

Forced swim test
The forced swim test is a well-characterized behavioural paradigm to define the level of depressive-related behaviour. The test relies upon the animal's tendency for survival, therefore to keep swimming; depressed animals will start floating earlier and be immobile for a longer time. The forced swim test exists of two parts: the induction phase and test phase. On the induction day rats are placed in a cylinder filled with water for 15 min, on the test day rats are placed in the cylinder for 5 min (Olivier et al., 2008). Time spent on swimming and floating (=psychomotor retardation) is measured.

***Sacrifice*:** 24 hours after the last behavioural test the rats on pharmacological treatment will be sacrificed by CO2.

Experiment 5: Pharmacological prevention of antidepressant-induced mania and aberrant glutamate neurotransmission
In this experiment we will test whether imipramine supplemented with an agent approved from human use that reduces glutamate neurotransmission prevents the imipramine-induced shift to mania. The procedure used in experiment 1 will be copied, but now in the presence of an imipramine supplement.
Treatment: To prevent the switch to mania during antidepressant treatment, male and female 5-HTT and DAT KO rats, and wild-type controls rats are first are 'treated' with vehicle (water) for 4 weeks, and the behavioural test battery as set out below is conducted. Then, the animals are treated with 1) imipramine (Sigma Aldrich), or 2) imipramine + N-acetylcysteine/Memantine/LY2140023 (one of these three drugs). Imipramine (10 mg/kg/day) will be administered via drinking water for 6 weeks, starting from the week 5 to 10 until the day of sacrifice. Imipramine will dissolve in the drinking water at concentrations of 180mg/L (Broitman and et al.). In the group that receives an imipramine supplementation, animals will receive N-acetylcysteine (800 mg/kg/day) (Otte; 2011, Gürer; 1998), Memantine 30 mg/kg/day (Ihalainen; 2011, Gao; 2011) or LY2140023 (30 mg/kg) (Lowe; 2012) via drinking water for 6 weeks.

***Behavioural testing*:** Depression- and mania-like behavior (see **Table 1** and tests described under experiment 1) will be measured during imipramine + N-acetylcysteine, imipramine + LY2140023 or imipramine + memantine treatment, starting 2 weeks after start of the treatment.

***Sacrifice*:** 24 hours after the last behavioural test, the rats on pharmacological treatment will be sacrificed by CO2.

References:
Baker, P. M., et al. "Lateral habenula integration of proactive and retroactive information mediates behavioral flexibility." *Neuroscience* 345 (2017): 89-98.
Benekareddy, Madhurima, et al. "Identification of a corticohabenular circuit regulating socially directed behavior." *Biological psychiatry* 83.7 (2018): 607-617.
Broitman, Susana T., and A. O. Donoso. "Effects of chronic imipramine and clomipramine oral administration on maternal behaviour and litter development." *Psychopharmacology* 56.1 (1978): 93-101.

Enkel T, Gholizadeh D, von Bohlen Und Halbach O, Sanchis-Segura C, Hurlemann R, Spanagel R, Gass P, Vollmayr B. Ambiguous-cue interpretation is biased under stress- and depression-like states in rats. *Neuropsychopharmacology*. 2010 Mar;35(4):1008-15.
Gao, Y., Payne, R. S., Schurr, A., Hougland, T., Lord, J., Herman, L., ... & El-Mallakh, R. S. (2011). Memantine reduces mania-like symptoms in animal models. *Psychiatry research*, *188*(3), 366-371.
Gürer, Hande, et al. "Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats." *Toxicology* 128.3 (1998): 181189.
Ihalainen, Jouni, et al. "Effects of memantine and donepezil on cortical and hippocampal acetylcholine levels and object recognition memory in rats." *Neuropharmacology* 61.5-6 (2011): 891-899.
Lowe, Stephen, et al. "Effects of a novel mGlu 2/3 receptor agonist prodrug, LY2140023 monohydrate, on central monoamine turnover as determined in human and rat cerebrospinal fluid." *Psychopharmacology* 219.4 (2012): 959-970.
Nada, M-B., et al. "The puzzle box as a simple and efficient behavioral test for exploring impairments of general cognition and executive functions in mouse models of schizophrenia." *Experimental neurology* 227.1 (2011): 42-52.
Niesink, Raymond JM, and J. M. Van Ree. "Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats." *Neuropharmacology* 28.4 (1989): 411-418.
Olivier, J. D. A., et al. "A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders." *Neuroscience* 152.3 (2008): 573-584.
Otte, David-Marian, et al. "N-acetyl cysteine treatment rescues cognitive deficits induced by mitochondrial dysfunction in G72/G30 transgenic mice." *Neuropsychopharmacology* 36.11 (2011): 2233.
Pellow, Sharon, et al. "Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat." *Journal of neuroscience methods* 14.3 (1985): 149-167.

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

Pilot: Most variability is seen in the depression-related tests. A power analysis based on depression-related measures in 5-HTT KO rats (Olivier, 2008) revealed an effect size of 0.62, making that at $\alpha=0.05$ and $1-\beta=0.8$, an N=10 is needed. For the 0A pilot study, we will use N=11 rats per genotype to conduct the behavioural tests. We will use both males and female animals all studies, as we do not know whether the imipramine-induced switch from depression to mania is sex dependent. It is possible that males and female display a different behavioural change after imipramine treatment. If so, data from the pilot cannot be generalized from one sex to the other. This we overcome by testing both sexes. We increase N=10 to N=11 to cover 10% dropout because our experiment will last for 10 weeks and it is quite long time for testing. There is for instance the possibility that one of the animals loses weight and reaches the human end point because water consumptive drops due to changing its taste with imipramine. This allows us to use the pilot not only as pilot, but also for proper statistics and publication.
Experiment 1 and 5, based on calculation mentioned above for behaviour, is estimated to require N=11 rats (N=10 + 10% dropout because of longitudinal design).
Experiment 1 and 5: After the pilot study we will be able to conduct a power analysis for experiment 1 and 5. For now we estimate that we need N=11, like for the pilot.

References:
Olivier JD, Van Der Hart MG, Van Swelm RP, Dederen PJ, Homberg JR, Cremers T, Deen PM, Cuppen E, Cools AR, Ellenbroek BA. A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. Neuroscience. 2008 Mar 27;152(3):573-84.

B. The animals

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

For experiment 0A adult male and female 5-HTT KO as knockout rats and 5-HTT WT as wild-type rats will be used.
For experiment 1 and 5, adult male and female 5-HTT KO, DAT KO as knockout rats and 5-HTT WT and DAT WT as wild-type rats will be used. These rats model 5-HTT and DAT polymorphisms that in humans have been found to be associated with bipolar disorder, and which show depression-like and mania-like phenotypes. A more detailed description is provided in the project proposal. We use both males and females because gender differences in the phenomenology and course of the illness have been reported among male and female with bipolar disorder. Some studies with clinical samples report that women with bipolar disorder experience fewer manic episodes and more episodes of depression compared to men with bipolar disorder (Taylor, 1987). Women have reportedly higher rates of bipolar depression and type II bipolar disorder (depression with hypomania), a greater likelihood of having depression precede mania or hypomania, are more likely to be hospitalized for mania and have a rapid□cycling course somewhat more often than men (Viguera, 2001). Sex differences in the phenomenology and course of bipolar disorder points to potential sex differences in underlying mechanisms, which are unknown up to date. And such sex differences could in turn influence the efficacy of interventions. Indeed, some studies about differences in the treatment response of male and female with bipolar disorder suggest that there may be sex differences in response to mood stabilizers (Kawa, 2005). Hence, findings in one sex may not generalize to the other sex. In this project we will test both male and female rats. The 'speed' of the antidepressant-induced switch to mania can be assessed through the mania-related tests. It is possible that mania is seen in the first test (motivation test) in some animals, but not others, while all animals show mania-like phenotypes in the subsequent mania-like tests. Or, it is possible that within the motivation test there is a delay in an increase in motivation. It is also possible that the level of mania differs per sex or that this is task dependent. The experiments will have to point this out. We use adult rats as there is a high prevalence of bipolar disorder in adulthood (Hamlat et al., 2016). Although depression can also emerge in adolescence, we focus on one age group here for feasibility. Indeed, the duration of behavioural tests is too long for a short developmental period such as adolescence. We will use both 5HTT WT and DAT KO WT rats because genetically they have different background (SERT line: Wistar Unilever; DAT line: Wistar Hanover) and we should compare each genetic line with own control group.

References:
Taylor, Michael Alan, and Richard Abrams. "Gender differences in bipolar affective disorder." *Journal of affective disorders* 3.3 (1981): 261-271.
Viguera, Adele C., Ross J. Baldessarini, and Leonardo Tondo. "Response to lithium maintenance treatment in bipolar disorders: comparison of women and men." *Bipolar Disorders*3.5 (2001): 245-252.

Kawa, Izabela, et al. "Gender differences in bipolar disorder: age of onset, course, comorbidity, and symptom presentation." *Bipolar disorders* 7.2 (2005): 119-125.
Hamlat, Elissa J., et al. "Assessment and Treatment of Bipolar Spectrum Disorders in Emerging Adulthood: Applying the Behavioral Approach System Hypersensitivity Model." *Cognitive and behavioral practice* 23.3 (2016): 289-299.

The following groups will be included in the pilot Experiment (0A):

Nr.	Group (n=11)	Treatment	Behaviour (see table 1, pp)
1	Male 5-HTT KO	Vehicle and Imipramine	All tests
2	Male 5-HTT WT	Vehicle and Imipramine	All tests
3	Female 5-HTT KO	Vehicle and Imipramine	All tests
4	Female 5-HTT WT	Vehicle and Imipramine	All tests

4x 11 = 44 rats

The groups in **experiment 1** will be as follow:

Nr.	Group (n=11)	Treatment	Behaviour (see table 1)
1	**Male 5-HTT KO**	**Vehicle and imipramine**	**Selection of tests**
2	Male 5-HTT WT	Vehicle and imipramine	Selection of tests
3	**Female 5-HTT KO**	**Vehicle and imipramine**	**Selection of tests**
4	Female 5-HTT WT	Vehicle and imipramine	Selection of tests
5	**Male DAT KO**	**Vehicle and imipramine**	**Selection of tests**
6	Male DAT WT	Vehicle and imipramine	Selection of tests
7	**Female DAT KO**	**Vehicle and imipramine**	**Selection of tests**
8	Female DAT WT	Vehicle and imipramine	Selection of tests

8 x 11 = 88 rats

The groups in **experiment 5** will be as follow:

Treatment and behavioural tests			
Nr.	**Group (n=11)**	**Treatment**	**Behaviour (see table 1)**
1	Male 5-HTT KO	Imipramine + vehicle (from week 5 to 10)	Selection of tests
2	Male 5-HTT KO	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests
3	Male 5-HTT WT	Imipramine + vehicle (from week 5 to 10)	Selection of tests
4	Male 5-HTT WT	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests
5	Female 5-HTT KO	Imipramine + vehicle (from week 5 to 10)	Selection of tests
6	Female 5-HTT KO	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests
7	Female 5-HTT WT	Imipramine + vehicle (from week 5 to 10)	Selection of tests
8	Female 5-HTT WT	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests
9	Male DAT KO	Imipramine + vehicle (from week 5 to 10)	Selection of tests
10	Male DAT KO	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests
11	Male DAT WT	Imipramine + vehicle (from week 5 to 10)	Selection of tests
12	Male DAT WT	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests
13	Female DAT KO	Imipramine + vehicle (from week 5 to 10)	Selection of tests
14	Female DAT KO	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests
15	Female DAT WT	Imipramine + vehicle (from week 5 to 10)	Selection of tests
16	Female DAT WT	Imipramine + N-acetylcysteine or LY2140023 or memantine (from week 5 to 10)	Selection of tests

16 x 11=176

Species	Origin	Maximum number of animals	Life stage
5-HTT KO	Own breeding	88	Adult (> PND 70)
5-HTT WT	Own breeding	88	Adult (> PND 70)
DAT KO	Own breeding	66	Adult (> PND 70)
DAT WT	Own breeding	66	Adult (> PND 70)

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
The rat is the best model to study human psychiatric disorders. Due to the complexity of depression as a disorder, it is impossible to use lower-order animals or organoids to perform this study. Animal models provide the advantage to control environmental conditions such as antidepressant treatment and potential other factors triggering the switch, and to reveal mechanisms in depth using invasive methods that ethically cannot be applied to humans. Cell-lines cannot be used because we need link neurochemical and behavior changes.

Reduction
The requested amount of animals is needed for statistical reliable conclusions and is the minimal group size one can work with. Furthermore, the same animals will be used for vehicle and drug treatment to obtain a high number of information, thereby leading to a minimal amount of animals needed. We will perform a pilot study to optimize our experimental procedures (e.g., imipramine administration, appropriate behavioural tests). Based on the pilot study, we will decide whether to continue with our main experiment as it was proposed or whether to adapt the procedures. When an adjustment is required we will submit an amendment.

Refinement

The experiments will be carried out with the least discomfort possible. For this reason, cage enrichment will be applied. Furthermore, imipramine and glutamatergic drugs are given through the drinking water, thereby minimizing the stress associated with imipramine treatment. To measure depression-like and mania-like behaviour the behavioural tests are necessary. However, based on a pilot study we will select the most relevant tests, thereby reducing the number of tests the animals will be exposed to. The analyses we propose cannot be performed without sacrificing animals. As usual, all efforts will be undertaken to minimize animal suffering during sacrifice. Only experienced researchers will sacrifice the animals.

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. The animals will receive imipramine via their drinking water; we chose this procedure to reduce the amount of stress for the animals. The drug will have no adverse physical effects on the animals' health. The animals are housed with minimally one other animal and will have a regular or enriched housing environment. Animals will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked daily to be able to detect Human End Point conditions and weighted once a week.

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.

There are no other studies that have performed experiments as proposed.

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.

Animals will be housed individually for a period of 3 days. This is necessary to enable proper measurements during the sleep/wake rhythm. It is for a short time and it will not have negative effects on them.

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?

[X] No > Continue with question I.
[] 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?

The rats will be subjected to a series of behavioural tests to measure depression-like and mania-like behaviour, which together produce psychological stress. However, there will no physical stress or damage due to behavioural testing. The rats are subjected to the forced swim test. In this test the water will be set at 24 0C to reduce stress, and the animals will be immediately removed from the water when their head is below the water surface. so acting immediately can reduce the stress and discomfort of animals during the test. Hence, this procedure does not exhaust the animals. Rats will receive an injection of amphetamine during open field test.

Explain why these effects may emerge.

The behavioural tests are needed to assess the level of depression-like and mania-like behaviour. Since bipolar disorder consists of a constellation of phenotype, one depression-like and one mania-like tests is not sufficient to draw conclusions on the shift in mood state. In the behavioural tests the rats are also exposed to novel environments, which will cause a level of stress similar to when their home cage is cleaned. All rats are socially housed, except during the sleep/wake rhythm test. All rats are housed with regular bedding and nesting material or even additional cage enrichment, which contributes to stress reduction.

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

All rats are socially housed (with exception during the sleep/wake rhythm test), with regular bedding and nesting material or even additional cage enrichment, which contributes to stress reduction.

J. Humane endpoints

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

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

The criteria to take the animal out of the experiments are based on human observation of factors known as clear symptoms of pain/stress/discomfort and defined for humane end point detection*. Weight loss of more than 15% in one/two days is considered as humane endpoints. General symptoms such as raised fur, hunched back (arched back), poor coat conditions, are also considered as humane endpoints after which the animals should be euthanized.
*Standard humane endpoints rodents: loss of body weight (> 15%), immobility, poor self-care, tremor, self-damage, abnormal body posture, convulsions, tumors, elephant teeth.

Indicate the likely incidence.

<10% for all experiments. We have explained in the section on group size estimation why animals may drop out. This has been accounted for in the group size calculation.

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).

Summary as follow:

Suffering	Rats	undergo	Number of animals	Experiment
Moderate	Male and female	Imipramine treatment, behaviour	44	Pilot 0A
Moderate	Male and female	Imipramine treatment, Behaviour	88	1
Moderate	Male and female	Pharmacological treatment, Behaviour	176	5
			Total= 308	

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

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

The aim of using animals in the experiments 0A, 1 and 5 is studying the different behaviors of rats related to depression and mania due to pharmacological treatment. Because the animals received pharmacological treatment, they are not suitable to function as surplus animals in experiments of other researchers. Therefore, we suggest to sacrifice the animals 24 hours after the last behavioural test by CO2 (method that is typically used if no biomaterials of the animals are needed).

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

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

[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 Ex vivo measurements

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.

In experiment 2, male and female 5-HTT and DAT KO rats and their wild-type controls will be treated with imipramine in the drinking water (or water control), for 3 weeks. 24 Hrs after the last treatment day rats are decapitated. Primary outcome parameters for experiment 2 are as follows: To study how serotonin and dopamine modulate physiologic glutamate homeostasis in the PFC, striatum and LHb, we will measure the expression of critical determinants of the glutamate synapse (NMDA receptors, AMPA receptors, metabotropic receptors; their main scaffolding proteins necessary to properly anchor the receptors at the membrane, the glutamate transporter GLT-1, and the astroglial Kir4.1 (a potassium channel mediating LHb burst firing), in the membrane (Hanford, 2016).

In experiment 3, male and female 5-HTT and DAT KO rats and their wild-type controls will be treated with imipramine in the drinking water (or water control), for 3 weeks. 24 Hrs after the last treatment day rats are decapitated. Primary outcomes parameters for experiment 3 are as follows:
By recording evoked transmission (eEPSC), we will measure how synaptic transmission involving both pre- and postsynaptic efficacy changes in relation to switches to mania-like behaviour. Furthermore, presynaptic release probability of glutamate following imipramine treatment will be assessed by paired-pulses stimulations (10 to 50 ms apart). We will also investigate how imipramine alters glutamatergic hyperexcitability in the LHb of 5-HTT KO and DAT KO, rats by changing postsynaptic AMPA/NMDA receptor function.

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

Experiment 2: Effect of antidepressant treatment on gene expression levels of glutamate system components
Male and female 5-HTT and DAT KO rats and their respective controls will be treated for 3 weeks and sacrificed for molecular analyses. This group will not be subjected to behaviour tests and microdialysis in order to avoid effects on the testing and surgery on gene expression. Because the rats are not behaviourally tested and subjected to microdialysis, treatment will last 3 weeks, which is sufficient for chronic treatment in rodents (Lee, 2010). Furthermore, we apply a cross-sectional design, because rats have to be sacrificed for ex-vivo molecular measurements (a longitudinal design as in experiment 1 and 4 is thus not possible)
Treatment: Rats will be treated with imipramine/vehicle for 3 weeks, and 24 hours after the last treatment day rats will be decapitated without anesthesia to avoids problems with gene expression changes. Brain will be rapidly removed from the skull, frozen on dry ice, and stored at -80 0C, until use for molecular analyses. After sacrificing the animals, we will investigate by Western blot analyses how serotonin and dopamine modulate physiologic glutamate homeostasis in the PFC, striatum and LHb.

Experiment 3: Ex vivo electrophysiology to elucidate the physiological characteristics of glutamatergic synapses due to antidepressant treatment
Male and female 5-HTT and DAT KO rats and their respective controls will be treated for 3 weeks and sacrificed for ex vivo electrophysiological recordings

Treatment: Rats will get imipramine (10 mg/kg/day) or vehicle for 3 weeks (emerging mania (Lee, H. J, 2010)). After three weeks, animals will be decapitated without anesthesia to prevent anesthesia effect on electrophysiological features of the brain (Rammes, 2009) and also keep the conditions comparable between experiments 2 and 3. Then the brains will be removed to continue with the electrophysiological study.

Electrophysiology recording:
To further test the overall hypothesis, we will perform whole cell patch-clamp experiments in acute slices of the LHb to better characterize pre- and postsynaptic glutamatergic transmission. Dissection and slicing will be performed as for extracellular recording. For recording, slices will be transferred in a submersion recording chamber perfused with warm and oxygenated aCSF (28°C, 2 ml/min) and mounted on an upright microscope equipped for infrared visualization and patch-clamp recording. LHb neurons will be voltage-clamped using Axopatch 200B (molecular devices). Glass pipettes with a resistance of 2–6 MΩ will be filled with an internal solution. mEPSCs will be recorded at -70 mV in the presence of 1 µM TTX, and the amplitude and the frequency will be analyzed using Mini Analysis software (Synaptosoft). Picrotoxin will be added to the aCSF to exclude GABAR-mediated inhibitory synaptic transmission. For recordings of evoked transmission (eEPSC), QX314 (5 mM) will be added to the internal solution and stria medullaris will be stimulated using twisted nickel-chrome electrodes. Presynaptic release probability will be assessed by paired-pulses stimulations (10 to 50 ms apart). To test whether there are alterations in the relative expressions of postsynaptic AMPA receptors with different permeabilities, we will measure the rectification index in the presence of spermine. Finally, NMDAR EPSCs will be recorded by stimulating the input stria medullaris fibre in a modified extracellular aCSF solution with the AMPAR blocker NBQX and 0 Mg2+.

References:
Lee, H. J., et al. "Chronic imipramine but not bupropion increases arachidonic acid signaling in rat brain: is this related to 'switching'in bipolar disorder?." Molecular psychiatry 15.6 (2010): 602.
Rammes, Gerhard, et al. "Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B subunit expression." Neuropharmacology 56.3 (2009): 626-636.

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

In experiment 2 we will have a treatment only group (without behaviour/microdialysis), for which previous 5-HTT KO molecular studies (Fumagalli, unpublished) N=8/group (effect size: 1.5339300; 1-b=0.8; a=0.05; N=8) is sufficient. In experiment 3 (electrophysiology measurements), we will focus on the LHb and perform whole cell patch-clamp recording. For this part of the project, 16 rats per group will be needed to reach 30 to 40 cells analyzed in each group. We estimate based on previous (5-HTT KO) electrophysiology studies (Miceli; 2017, Park; 2017) that N=16 (power: effect size: 1.2128146, 1-b=0.8, a=0.05) will allow to reach significance in group comparisons. Slices will be excluded when they don't show reliable baseline signal, or when the values are above 3 standard deviations.

References:
Miceli, Stéphanie, et al. "Reduced inhibition within layer IV of sert knockout rat barrel cortex is associated with faster sensory integration." Cerebral Cortex 27.2 (2017): 933-949.

Homberg JR, Olivier JD, Smits BM, Mul JD, Mudde J, Verheul M, Nieuwenhuizen OF, Cools AR, Ronken E, Cremers T, Schoffelmeer AN, Ellenbroek BA, Cuppen E. Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. Neuroscience. 2007 Jun 8;146(4):1662-76.
Verheij MM, Karel P, Cools AR, Homberg JR. Reduced cocaine-induced serotonin, but not dopamine and noradrenaline, release in rats with a genetic deletion of serotonin transporters. Eur Neuropsychopharmacol. 2014 Nov;24(11):1850-4.
Park H, Cheon M, Kim S, and Chunga C. Temporal variations in presynaptic release probability in the lateral habenula. Sci Rep. 2017; 7: 40866.

B. The animals

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

For experiment 2 and 3, adult male and female 5-HTT KO, DAT KO as knockout rats and 5-HTT WT and DAT WT as wild-type rats will be used. These rats model 5-HTT and DAT polymorphisms that in humans have been found to be associated with bipolar disorder, and which show depression-like and mania-like phenotypes (see pp). We use male and female rats since bipolar disorder is seen in both males and females but with some qualitative differences (e.g. females show mood shifts sooner) (Arnold LM, 2003). Using both sexes we can investigate why. See DAP 1 for further explanation on sex differences we may observe. We use adult rats as there is a high prevalence of bipolar disorder in adulthood (Hamlat, 2016). Although depression can also emerge in adolescence, we focus on one age group here for feasibility. Indeed, the duration of behavioural tests is too long for a short developmental period such as adolescence.

References:

Arnold, Lesley M. "Gender differences in bipolar disorder." The Psychiatric Clinics of North America 26.3 (2003): 595-620.
Hamlat, Elissa J., et al. "Assessment and Treatment of Bipolar Spectrum Disorders in Emerging Adulthood: Applying the Behavioral Approach System Hypersensitivity Model." Cognitive and behavioral practice 23.3 (2016): 289-299.

The groups in **experiment 2** will be as follow:

Treatment without behaviour/microdialysis	
Imipramine treatment group (n=8)	Vehicle treatment group (n=8)
Male 5-HTT KO	Male 5-HTT KO
Male 5-HTT WT	Male 5-HTT WT
Female 5-HTT KO	Female 5-HTT KO
Female 5-HTT WT	Female 5-HTT WT
Male DAT KO	Male DAT KO
Male DAT WT	Male DAT WT
Female DAT KO	Female DAT KO
Female DAT WT	Female DAT WT

16 x 8 = 128 rats

The animal groups in **experiment 3** (electrophysiology measurement) will be as follow:

Nr.	Group (n=16)	Treatment	Experiment
1	Male 5-HTT KO	imipramine	Electrophysiology recording
2	Male 5-HTT WT	imipramine	Electrophysiology recording
3	Female 5-HTT KO	imipramine	Electrophysiology recording
4	Female 5-HTT WT	imipramine	Electrophysiology recording
5	Male DAT KO	imipramine	Electrophysiology recording
6	Male DAT WT	imipramine	Electrophysiology recording
7	Female DAT KO	imipramine	Electrophysiology recording
8	Female DAT WT	imipramine	Electrophysiology recording
9	Male 5-HTT KO	vehicle	Electrophysiology recording
10	Male 5-HTT WT	vehicle	Electrophysiology recording
11	Female 5-HTT KO	vehicle	Electrophysiology recording
12	Female 5-HTT WT	vehicle	Electrophysiology recording
13	Male DAT KO	vehicle	Electrophysiology recording
14	Male DAT WT	vehicle	Electrophysiology recording
15	Female DAT KO	vehicle	Electrophysiology recording
16	Female DAT WT	vehicle	Electrophysiology recording

16 x 16 = 256 rats

Species	Origin	Maximum number of animals	Life stage
5-HTT KO	own breeding	96	Adult (> PND 70)
5-HTT WT	own breeding	96	Adult (> PND 70)
DAT KO	own breeding	96	Adult (> PND 70)
DAT WT	own breeding	96	Adult (> PND 70)

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
The rat is the best model to study human psychiatric disorders. Due to the complexity of bipolar disorder, it is impossible to use lower-order animals or organoids to perform this study. Animal models provide the advantage to control environmental conditions such as antidepressant treatment and potential other factors triggering the switch, and to reveal mechanisms in depth using invasive methods that ethically cannot be applied to humans.

Reduction
The requested amount of animals is needed for statistical reliable conclusions and is the minimal group size one can work with.

Refinement
The experiments will be carried out with the least discomfort possible. For this reason, cage enrichment will be applied. Furthermore, imipramine is given through the drinking water, thereby minimizing the stress associated with imipramine treatment. The analyses we propose cannot be performed without sacrificing animals. As usual, all efforts will be undertaken to minimize animal suffering during sacrifice. Only experienced researchers will sacrifice the animals.

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

The discomfort which our rats will be exposed to, is limited to the absolute minimum necessary to answer our research questions. The animals will receive imipramine via their drinking water; we chose this procedure to reduce the amount of stress for the animals. The drug will have no adverse physical effects on the animals' health. The animals are housed with minimally one other animal and will have a regular or enriched housing environment.
Animals will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked daily to be able to detect Human End Point conditions and weighted once a week.

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.

There are no other studies that have performed experiments as proposed

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?

F. Accommodation and care

[X] No

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

G. Location where the animals procedures are performed

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

[X] No > Continue with question H.

[] Yes > Describe this establishment.

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

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.

[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

I. Other aspects compromising the welfare of the animals

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

None. Rats are treated through the drinking water, and are further not handled.

Explain why these effects may emerge.

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

All rats are socially housed, with regular bedding and nesting material or even additional cage enrichment, which contributes to stress reduction.

J. Humane endpoints

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

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

The criteria to take the animal out of the experiments are based on human observation of factors known as clear symptoms of pain/stress/discomfort and defined for humane end point detection*. Weight loss of more than 15% is considered as humane endpoints. Also general symptoms such as raised fur, hunched back (arched back), poor coat conditions, are also considered as humane endpoints after which the animals should be euthanized. We will contact a veterinarian if there is doubt.
*Standard humane endpoints rodents: loss of body weight (> 15%), immobility, poor self-care, tremor, self-damage, abnormal body posture, convulsions, tumors, elephant teeth.

Indicate the likely incidence.

<2%

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).

Summary as follow:

Suffering	Rats	undergo	Number of animals	Experiment
Mild	Male and female	Imipramine treatment and decapitation	128	2
Mild	Male and female	Imipramine treatment and decapitation	256	3
			Total=384	

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.

Experiment 2: Rats will be sacrificed by decapitation without anesthesia for molecular studies such as expression of critical determinants of the glutamate synapse (NMDA receptors, AMPA receptors, metabotropic receptors; their main scaffolding proteins necessary to properly anchor the receptors at the membrane, the glutamate transporter GLT-1, and the astroglial Kir4.1) in the membrane. Experiment 3: Rats will be decapitated without anesthetized for electrophysiological recordings.

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

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

In experiment 2 and 3, rats will be sacrificed by decapitation without anesthesia for molecular studies and electrophysiological recordings respectively. We do not use anesthesia, as anesthesia might interfere with the molecular and and electrophysiological measurements. Indeed, anesthetics have an effect on glutamate neurotransmission (Rammes, 2009).
Reference:
Rammes, Gerhard, et al. "Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B subunit expression." Neuropharmacology 56.3 (2009): 626-636.

[] 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 3	Type of animal procedure Surgery

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.

In the pilot study (experiment 0B), we will use surplus rats for the stereotaxic surgery in order to find the coordinates of the striatum, LHb and mPFC in the brain for the microdialysis experiment.

In experiment 4, male and female 5-HTT and DAT KO rats and their wild-type controls will be treated with imipramine in the drinking water (or water control), for 3 weeks. 24 Hrs after the last treatment day rats are decapitated. Primary outcome parameters for experiment 4 are as follows: To study dopamine and serotonin (as well as their metabolites), and ii) glutamate (as well as GABA and glutamine) levels after treatment with imipramine.

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

Experiment 0: pilot study
Prior to the main experiment, a pilot study (experiment 0B) will be executed with surplus animals. By practicing surgeries on these rats, we can find the proper coordinates of the striatum, LHb and mPFC in the brain and the targeting of two areas in one animals, for the microdialysis experiment.

Experiment 4: Effect of antidepressant treatment on neurotransmitters level
To study the neurotransmitter changes during the antidepressant-induced switch to mania we will conduct a stereotactic surgery to place guide cannulas in the striatum, LHb and/or mPFC, treat the rats with vehicle for 1 week, measure neurotransmitter levels by in vivo microdialysis, subject the rats to imipramine for 3 weeks, and repeat the microdialysis experiment. Through this longitudinal design we will be able to assess changes in neurotransmitter levels in the same animals.

Treatment: Male and female 5-HTT and DAT KO rats, and wild-type controls, will be treated with vehicle (water), and subsequently imipramine. Imipramine (10 mg/kg/day) will be administered via drinking water for 2 weeks, starting from week five to eight. Rats will undergo a surgery to unilaterally place guide cannulas into the striatum and LHb in the same animal. In other groups of animals, we will measure neurotransmitters in the medial PFC (mPFC) and LHb by unilaterally implanting two guide cannulas in the regions mentioned. The rats thus get two cannulas in their brain. This 1. saves animals, 2. does not cause more suffering for animals since it will be done during one surgery, and 3. Allows investigation of correlations between neurotransmission changes in the two areas. We will study these three areas because the PFC-striatum-LHb circuitry is expected to play key role in mania. Prefrontal glutamate signaling mediates top-down control over subcortical areas processing reward (e.g. striatum) and sociability/emotion (e.g. LH) (Benekareddy, 2018). In addition, LHb receives input from both the mPFC and striatum, raising the possibility that the LHb acts as a relay between forebrain and midbrain (Baker, 2017) and this is a reason we include this area in both microdialysis

groups. After recovery, animals will be treated with water (vehicle) for one week and will be subjected to an in vivo microdialysis study in which we assess dopamine, serotonin and glutamate levels in vivo in the LHb, mPFC and nucleus accumbens. Subsequently, the rats will be treated with imipramine for 3 weeks and the in vivo microdialysis experiment will be repeated. We measure off-line levels of i) dopamine and serotonin (as well as their metabolites), and ii) glutamate (as well as GABA and glutamine). The microdialysis probe is inserted into guide cannulas in the evening before the test day. On the test day baseline levels of neurotransmitter are measured during 3-4 hours (15 min samples). Samples are split in two, analyzed by high pressure liquid chromatography using two different columns, one for the monoamines and one for the amino acids. These monoamine levels are primary outcome. After the last microdialysis experiment, rats will be sacrificed by perfusion under deep anesthesia with pentobarbital (i.p), to obtain fixed brains for validation of the placement of the cannulas site.

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

In 0B experiment will we will use 20 rats in total; 10 rats to practice cannula placement in the mPFC and LHb, and 10 rats to practice cannula placement in the striatum and LHb (see next section for explanation for the combination of these regions).
In experiment 4 (microdialysis), we increase the group size to N=10/genotype and sex to be able to remove animals from the experiment when rats drop-out because of recover insufficiently from surgery, or guide cannulas are incorrectly positioned. More concretely, we expect that we need N=8 rats for microdialysis based on our previous microdialysis experiments (Verheij et al., 2014; Homberg et al., 2007) and the aim to find not only bold changes in serotonin and dopamine levels, but also more subtle differences in glutamate levels. When having groups of N=10, we can cover a drop-out of 20%. We estimate a drop-out of 20%, because we place two cannula in the brain of each rat. With two cannula, there is increased risk that one of the cannulas is wrongly placed in the brain (chance 5%). There is a small chance for an infection or loss of a cannula (5%). Finally, there is moderate chance (10%) that the dialysis probe is leaking during the experiment, or that, due to movements of the animals (especially in case of the hyperactive DAT KO rats), the rats are detatched from the tubings of the mcirodialysis system (10%). Microdialysis is a very delicate technique, but also very worthful. In this light, the 20% dropout is not high, and in line with our experiences.

References:
Miceli, Stéphanie, et al. "Reduced inhibition within layer IV of sert knockout rat barrel cortex is associated with faster sensory integration." Cerebral Cortex 27.2 (2017): 933-949.

Homberg JR, Olivier JD, Smits BM, Mul JD, Mudde J, Verheul M, Nieuwenhuizen OF, Cools AR, Ronken E, Cremers T, Schoffelmeer AN, Ellenbroek BA, Cuppen E. Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. Neuroscience. 2007 Jun 8;146(4):1662-76.
Verheij MM, Karel P, Cools AR, Homberg JR. Reduced cocaine-induced serotonin, but not dopamine and noradrenaline, release in rats with a genetic deletion of serotonin transporters. Eur Neuropsychopharmacol. 2014 Nov;24(11):1850-4.

Park H, Cheon M, Kim S, and Chunga C. Temporal variations in presynaptic release probability in the lateral habenula. Sci Rep. 2017; 7: 40866.

B. The animals

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

For experiment 0B surplus 5-HTT rats will be used.
For experiment 4, adult male and female 5-HTT KO, DAT KO as knockout rats and 5-HTT WT and DAT WT as wild-type rats will be used. These rats model 5-HTT and DAT polymorphisms that in humans have been found to be associated with bipolar disorder, and which show depression-like and mania-like phenotypes (see pp). We use male and female rats since bipolar disorder is seen in both males and females but with some qualitative differences (e.g. females show mood shifts sooner) (Arnold LM, 2003). Using both sexes we can investigate why. See DAP 1 for further explanation on sex differences we may observe. We use adult rats as there is a high prevalence of bipolar disorder in adulthood (Hamlat, 2016). Although depression can also emerge in adolescence, we focus on one age group here for feasibility. Indeed, the duration of behavioural tests is too long for a short developmental period such as adolescence.

References:
Arnold, Lesley M. "Gender differences in bipolar disorder." The Psychiatric Clinics of North America 26.3 (2003): 595-620.
Hamlat, Elissa J., et al. "Assessment and Treatment of Bipolar Spectrum Disorders in Emerging Adulthood: Applying the Behavioral Approach System Hypersensitivity Model." Cognitive and behavioral practice 23.3 (2016): 289-299.

The groups in 0B pilot study will be as follow:

Nr.	Group (n=10)	Behaviour (see table 1, pp)
1	surplus	Practice stereotactic surgery mPFC + LHb
2		Practice stereotactic surgery striatum + LHb

2 x 10 = 20 rats

The animal groups in **experiment 4** (microdialysis) will be as follow:

Nr.	Group (n=10)	Treatment	Microdialysis
1	Male 5-HTT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
2	Male 5-HTT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)
3	Male 5-HTT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
4	Male 5-HTT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)
5	Female 5-HTT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
6	Female 5-HTT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)
7	Female 5-HTT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
8	Female 5-HTT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)
9	Male DAT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
10	Male DAT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)
11	Male DAT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
12	Male DAT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)
13	Female DAT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
14	Female DAT KO	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)
15	Female DAT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (the striatum and LHb)
16	Female DAT WT	Vehicle (first week after surgery) Imipramine (week 2-4)	Microdialysis (mPFC and LHb)

16 x 10 = 160 rats

Species	Origin	Maximum number of animals	Life stage
Surplus	own breeding	20	Adult (> PND 70)
5-HTT KO	own breeding	40	Adult (> PND 70)
5-HTT WT	own breeding	40	Adult (> PND 70)
DAT KO	own breeding	40	Adult (> PND 70)
DAT WT	own breeding	40	Adult (> PND 70)

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

The rat is the best model to study human psychiatric disorders. Due to the complexity of bipolar disorder, it is impossible to use lower-order animals or organoids to perform this study. Animal models provide the advantage to control environmental conditions such as antidepressant treatment and potential other factors triggering the switch, and to reveal mechanisms in depth using invasive methods that ethically cannot be applied to humans.

Reduction
The requested amount of animals is needed for statistical reliable conclusions and is the minimal group size one can work with. Furthermore, the same animals will be used for vehicle and drug treatment to obtain a high number of information, thereby leading to a minimal amount of animals needed. Lower animals cannot be used because the surgery procedures are relatively complicated leading to an unacceptable high exclusion of animals (in which the position of the cannula might be wrong). We will perform a pilot study to optimize our experimental procedures (coordinations for stereotactic surgery).

Refinement
The experiments will be carried out with the least discomfort possible. For this reason, cage enrichment will be applied. Furthermore, imipramine is given through the drinking water, thereby minimizing the stress associated with imipramine treatment. The analyses we propose cannot be performed without sacrificing animals.

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

The discomfort which our rats will be exposed to, is limited to the absolute minimum necessary to answer our research questions. The animals will receive imipramine via their drinking water; we chose this procedure to reduce the amount of stress for the animals. The drug will have no adverse physical effects on the animals' health. The animals are housed with minimally one other animal and will have a regular or enriched housing environment.
Animals will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked daily to be able to detect Human End Point conditions and weighted once a week. Furthermore, due to stress after surgery, rats may be more afraid to human contact. However, after handling, these rats show normal behaviour again. Surgery for microdialysis will be performed under isoflurane anesthesia. In addition, lidocaine spray will be applied to the skin of the head. Possible bleeding will be stopped using epinephrine injection. 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, the animal's condition will be monitored on a daily basis.

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.

There are no other studies that have performed experiments as proposed

Accommodation and care

F. Accommodation and care

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

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

G. Location where the animals procedures are performed

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

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

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

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

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

H. Pain and pain relief

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

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

Surgery will be performed under isoflurane anesthesia. In addition, Lidocaine spray will be applied to the skin of the head. Possible bleeding will be stopped using epinephrine pellets. Before surgery and 24 hours after surgery, rats will be subcutaneously injected with Rimadyl (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, the animal's condition will be monitored on a daily basis. The rats will receive an overdose of pentobarbital prior to the perfusion procedure, decreasing their stress and pain levels as much as possible.

I. Other aspects compromising the welfare of the animals

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

The rats will be subjected to surgery which produce physical and psychological stress.

Explain why these effects may emerge.

The rats will be subjected to surgery which produce physical and psychological stress.

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

All rats are socially housed, with regular bedding and nesting material or even additional cage enrichment, which contributes to stress reduction.

J. Humane endpoints

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

[] No > Continue with question K.

[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

The criteria to take the animal out of the experiments are based on human observation of factors known as clear symptoms of pain/stress/discomfort and defined for humane end point detection*. Weight loss of more than 15% is considered as humane endpoints. Also general symptoms such as raised fur, hunched back (arched back), poor coat conditions, are also considered as humane endpoints after which the animals should be euthanized. Due to the longitudinal design, surgeries and misplacement of cannula additional endpoint such as losing of the cannula is taken into account. We will contact a veterinarian if there is doubt.
*Standard humane endpoints rodents: loss of body weight (> 15%), immobility, poor self-care, tremor, self-damage, abnormal body posture, convulsions, tumors, elephant teeth.

Indicate the likely incidence.

The incidence is <20% at max. We have in the section on group size estimation explained why animals may drop out. This has been accounted for in the group size calculation.

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).

Summary as follow:

Suffering	Rats	undergo	Number of animals	Experiment
Moderate	Male and female	Surgery and perfusion	20	Pilot 0B
Moderate	Male and female	Imipramine treatment, Surgery and perfusion	160	5

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.

Experiment 0B and 5: Rats will be sacrificed by perfusion under deep anesthesia with pentobarbital (i.p), to obtain fixed brains for validation of the placement of the cannulas site.

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

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

[X] Yes

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

1 Algemene gegevens

1.1	Titel van het project	De glutamaterge mechanismen die ten grondslag liggen aan mania geïnduceerd door antidepressiva
1.2	Looptijd van het project	9-7-2019-31-5-2023
1.3	Trefwoorden (maximaal 5)	Bipolaire stoornis, glutamaat, rat, gedrag, antidepressivum

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

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

3 Projectbeschrijving

3.1	Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)	Bipolaire stoornis is lastig te behandelen omdat het wordt gekenmerkt door een afwisseling van tegenovergestelde stemmingen, namelijk depressie en mania. Patienten die voor depressie worden behandeld met antidepressiva kunnen doorschieten naar mania. De mechanismen in de hersenen die hier aan ten grondslag liggen zijn niet bekend. Het doel van dit project is om deze mechanismen op te helderen. Hierbij richten wij ons op de neurotransmitter glutamaat, omdat humane studies aangeven dat bipolaire stoornis gepaard gaat met verhoogde glutamaat niveaus. Glutamaat niveaus worden gemeten door middel van een techniek 'microdialyse'. Dit gebeurt in het levende dier. Ook wordt het glutamaat systeem fysiologisch en moleculair onderzocht in hersenmateriaal van gedode dieren. Het ophelderen van deze mechanismen kan bijdragen aan de ontwikkeling van een combinatie medicatie om deze verschuiving te voorkomen. Het tweede doel van dit project is om op basis van de mechanismen die we in het eerste deel van het project ontdekken de antidepressivum behandeling te combineren met een ander medicijn om de verschuiving naar mania te voorkomen.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	Bipolaire stoornis is een emstige hersenziekte dat gepaard gaat met een hoge mate van last voor zowel de patient als de omgeving en maatschappij. Alhoewel er behandelingen beschikbaar zijn voor bipolaire stoornissen, zijn deze nog suboptimaal. Een probleem is dat bij de behandeling van het ene symptoom (bijv. depressie) een ander symptoom (bijv. mania) kan ontstaan. In dit project wordt het onderliggende mechanisme onderzocht (wetenschappelijke relevantie), om toe te werken naar een medicatie die een deze verschuiving kan voorkomen (maatschappelijke relevantie). Dit project is onderdeel van een consortium waar andere partners ook bijdragen aan het begrijpen van de mechanismen, en deze mechanismen vertalen naar de patient.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	In dit project maken we gebruik van mannelijke en vrouwelijke ratten. We zullen maximaal 872 ratten gebruiken.
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	De dieren worden blootgesteld aan een batterij van gedragstesten om depressie-achtig en mania-achtig gedrag te meten. Dit gaat gepaard met psychologische stress. De dieren zullen ook geopereerd worden, wat gepaard gaat met wondpijn. Ook het bijkomen uit narcose kan ongerief met zich mee brengen.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Mild voor 44 % van de dieren, en matig voor 56 % van de dieren.

3.6	Wat is de bestemming van de dieren na afloop?	De dieren worden na het onderzoek gedood. De hersenen van de dieren zullen gebruikt worden om vast te stellen of de operatie goed gelukt was, en om fysiologische en moleculaire metingen te doen op de hersenen.

4 Drie V's

4.1	**Vervanging**Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Omdat complex gedrag niet in lagere diersoorten of hersenen in een schaaltje gemeten kunnen worden zijn de ratten niet vervangbaar. Ook is het niet mogelijk om de metingen in mensen te verrichten, omdat het ethisch niet toelaatbaar is om hersenoperaties voor onderzoek te verrichten, en hersen materiaal te verkrijgen voor fysiologische en moleculaire metingen.
4.2	**Vermindering**Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	Het aantal aangevraagd dieren is minimaal nodig om statistisch verantwoorde conclusies te kunnen trekken uit de experimenten. Door combinatie van gedragsmetingen en in vivo metingen van glutamaat niveaus in de hersenen kan er uit een dier extra veel data verkregen worden.
4.3	**Verfijning**Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Het gebruik van lagere diersoorten dan de rat is niet mogelijk vanwege de complexiteit van psychiatrische stoornissen. Ook kunnen mensen niet gebruikt worden voor het onderzoek, omdat het onethisch is om in het humane brein chemische en moleculaire processen te bestuderen. De rat biedt de mogelijk om zowel complex gedrag te meten, als gedetailleerde hersen mechanismen te begrijpen
4.4	Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	De experimenten worden uitgevoerd door getrainde onderzoekers om de juiste uitvoering te garanderen en ongerief te beperken. De dieren worden behandeld met een antidepressivum via het drinkwater, waardoor injectie stress wordt voorkomen. Verdovende middelen en pijnstilling zullen worden toegediend rondom de hersenoperatie en opoffering. De operaties en opofferingen zullen plaatsvinden in een aparte ruimte, zodat de overige dieren hier geen stress van ervaren. De dieren zullen dagelijks aandachtig worden gemonitord, met name in de dagen na de hersenoperatie.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	

DEC-advies AVD1030020197744

A. Algemene gegevens over de procedure

1. Aanvraagnummer: AVD1030020197744 / 2019-0005
2. Titel van het project: Unveiling the mechanism(s) underlying the switch to mania during antidepressant treatment: The role of glutamate
3. Titel van de NTS: De glutamaterge mechanismen die ten grondslag liggen aan manie geïnduceerd door antidepressiva

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

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

6. Adviestraject (data dd-mm-jjjj):
   - ☒ ontvangen door DEC: 05-04-2019
   - ☐ aanvraag compleet
   - ☒ in vergadering besproken: 09-04-2019
   - ☐ anderszins behandeld
   - ☒ termijnonderbreking(en) van 15-04-2019 tot 01-05-2019
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   - ☒ aanpassing aanvraag: 01-05-2019
   - ☒ advies aan CCD: 15-05-2019

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

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

9. Correspondentie met de aanvrager:
   - Datum vragen: 15-04-2019
   - Datum antwoorden: 01-05-2019
   - Gestelde vragen en antwoorden:

Project Proposal:

– 3.2: De commissie zou graag zien dat u bij 3.2 eerst het hoofddoel van het project noemt, voordat u de subdoelen beschreven in experimenten 1 tot 5 noemt.
Antwoord: Bipolar Disorder (BD) is generally characterized by episodes of mania, hypomania, and depression. A well-known clinical feature of BD involves the switch from depression to mania during antidepressant treatment. The mechanisms underlying this switch are still elusive. Accordingly, our project will address the neurobiological mechanisms underlying the switch to mania during imipramine antidepressant treatment by taking advantage of the availability of two animal models, the serotonin transporter knockout (KO) rat and the dopamine transporter KO rat models. We hypothesize that the neurotransmitters serotonin and dopamine increase levels of the neurotransmitter glutamate in a brain circuitry that deals

with mood states like mania. Our study will provide a proof-of-principle of the glutamatergic mechanisms underlying mania by integrating behavioural, molecular, electrophysiological techniques.

– 3.4.1, 2B (bijlage), 2D (bijlage): De onderbouwing waarom u zowel mannen als vrouwen bestudeert ontbreekt. Dat zowel mannen als vrouwen bipolair kunnen zijn, zou er ook voor kunnen pleiten gemengde groepen te gebruiken. Het gebruikt van beide geslachten als aparte experimentele groepen verdubbelt het aantal dieren.
Daar moet een goede wetenschappelijke onderbouwing voor worden gegeven. De zinsnede in de bijlages vraag 2D, "we are required to use both males and females..." is niet onderbouwd. De commissie verzoekt u elders in de aanvraag (bij de strategie – 3.4 – in het project en 2B in de DAP) helder te beargumenteren waarom dit noodzakelijk is.
Antwoord: We are required to use both males and females because gender differences in the phenomenology and course of the illness have been reported among male and female with bipolar disorder. Some studies with clinical samples report that women with bipolar disorder experience fewer manic episodes and more episodes of depression compared to men with bipolar disorder (Taylor, 1987). Women have reportedly higher rates of bipolar depression and type II bipolar disorder (depression with hypomania), a greater likelihood of having depression precede mania or hypomania, are more likely to be hospitalized for mania and have a rapid-cycling course somewhat more often than men (Viguera, 2001). Sex differences in the phenomenology and course of bipolar disorder points to potential sex differences in underlying mechanisms, which are unknown up to date. And such sex differences could in turn influence the efficacy of interventions. Indeed, some studies about differences in the treatment response of male and female with bipolar disorder suggest that there may be sex differences in response to mood stabilizers (Kawa, 2005). Hence, findings in one sex may not generalize to the other sex. In this project we will test both male and female rats. The 'speed' of the antidepressant-induced switch to mania can be assessed through the mania-related tests. It is possible that mania is seen in the first test (motivation test) in some animals, but not others, while all animals show mania-like phenotypes in the subsequent mania-like tests. Or, it is possible that within the motivation test there is a delay in an increase in motivation. It is also possible that the level of mania differs per sex or that this is task dependent. The experiments will have to point this out. Since males and females have different speed in switching to mania and females with bipolar disorder show mood shifts sooner than men (Arnold LM, 2003), we cannot use them as mix groups. Additionally, Differences in underlying mechanisms and intervention effects cannot be revealed by mixing sexes.
Also, it is generally not accepted in preclinical research to mix sexes. Reviewers would ask to test both sexes separately with sufficient power. When mixing sexes in a group, each sex would have insufficient power for statistical analyses. We will need ultimately more animals if we set up experiments wrongly (sexes mixed; requiring total replication of the experiment), than when we do it right from the beginning (two sexes, each with sufficient power) according to the knowledge there are sex differences and scientific standard.

References:
Taylor, Michael Alan, and Richard Abrams. "Gender differences in bipolar affective disorder." Journal of affective disorders 3.3 (1981): 261-271.
Viguera, Adele C., Ross J. Baldessarini, and Leonardo Tondo. "Response to lithium maintenance treatment in bipolar disorders: comparison of women and men." Bipolar Disorders3.5 (2001): 245-252.
Kawa, Izabela, et al. "Gender differences in bipolar disorder: age of onset, course, comorbidity, and symptom presentation." Bipolar disorders 7.2 (2005): 119-125.
Arnold, Lesley M. "Gender differences in bipolar disorder." The Psychiatric Clinics of North America 26.3 (2003): 595-620.

– 3.4.2: De commissie ziet dat u de "forced swim test" als uitleesparameter voor depressie gebruikt. Het ongerief van de test wordt volgens de richtlijn EU2010/64 aangeduid als ernstig. U dient dan goed te onderbouwen waarom deze test noodzakelijk is en de ongeriefinschatting aan te passen. De commissie heeft overigens ernstige twijfels over de waarde van deze test in deze context (Molendijk ML, De Kloet R (2015): Immobility in the forced swim test is adaptive and does not reflect depression, Psychoneuroendocrinology, 62: 389-391).

Antwoord: The forced swim test (FST) is one of the most commonly used tests to assess depressive-like behavior in animal models of psychiatric disorders. The behavioral and biochemical characteristics of animals in a state of learned helplessness produced by a period of inescapable swimming during the FST have led some investigators to believe this condition itself provides a useful animal model of depression (Bogdanova, 2013). The FST is widely used in basic research and the pharmaceutical screening of potential antidepressant treatments. In our study, we will use FST for measuring depression-like behaviour in rats which will be treated with antidepressant imipramine because first, it involves the exposure of the animals to stress, which was shown to have a role in the tendency for major depression. Moreover, depression is often viewed as a lack of ability to handle with stress. Second, pharmacological treatment with antidepressants prior to the test has been shown to reduce immobility in the FST. Therefore, it is often used as a screening assay for novel compounds with potential antidepressant properties. Additionally, the FST has been shown to share some of the factors that are influenced or altered by depression in humans, such as changes in food consumption, sleep abnormalities and drug-withdrawal-induced anhedonia. This is also the reason why this test is sometimes used to evaluate depressive-like behavior, with increase or decrease in basal immobility (Yankelevitch, 2015; Detke, 1997). Against the paper of Molendijk and De Kloet there are many that successfully use the FST, in studies with translational data towards humans. For instance, the results from a study about differences in responses to different classes of antidepressants in the FST between adult and juvenile rats, reflects the clinical experience with these antidepressants in the human pediatric depression (Reed, 2008). Regarding the discomfort, it is mentioned in E2010/64 that animals are exhausted. As described in section, this is not what we do. As mentioned in section H (now I) of DAP1 "The rats are subjected to the forced swim test. In this test the water will be set at 24 0C to reduce stress, and the animals will be immediately removed from the water when their head is below the water surface. So acting immediately can reduce the stress and discomfort of animals during the test. Hence, this procedure does not exhaust the animals".

Hence, the FST as we will conduct does not correspond to the description in E2010/64. Furthermore, we find it unclear what the discomfort classification of this test (and the learned helplessness test) is based on. No literature or other source is mentioned. In science we have to 'open', but this guideline is not. It is more important that the animal suffering corresponds to what is happening in reality, we believe, and the animal suffering in these stress tests is totally not in line with the other conditions mentioned in E2010/64 where animals die or have permanent damage.

References:

Bogdanova, Olena V., et al. "Factors influencing behavior in the forced swim test." Physiology & behavior 118 (2013): 227-239.

(Yankelevitch-Yahav, Roni, et al. "The forced swim test as a model of depressive-like behavior." JoVE (Journal of Visualized Experiments) 97 (2015): e52587.)

Detke, Michael J., Jennie Johnson, and Irwin Lucki. "Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression." Experimental and clinical psychopharmacology 5.2 (1997): 107.

Reed, Abbey L., et al. "Juvenile rats in the forced-swim test model the human response to antidepressant treatment for pediatric depression." Psychopharmacology 197.3 (2008): 433-441.

– 3.4.2: Er wordt een aantal gedragstesten achter elkaar uitgevoerd. Hoe beïnvloeden deze testen elkaar? De HTT-5 KO rat vertoont een manisch-depressief beeld. Kunnen de gebruikte gedragstesten van invloed zijn op de uitkomsten afhankelijk van of de rat depressief of manisch is aan het begin van zo'n test?
Antwoord: We will use a number of behavioral tests in the same animal and a major strength of our approach is that conclusions will not rely on just one test for depression and one for mania. The animals will be tested in order of increasing stress, so in that case the behavioural tests related to stress and depression will have less influence on each other. Hence, we expect limited carry-over effects. The alternative is that each test will be conducted in separate animals. This 1. Increases the number of rats needed, and 2. Lacks within-animal information about the switch from depression-like behaviour to mania-like behaviour we aim to study. The only way to measure the switch is to conduct the proposed tests in the same animals.

– 3.4.2: Bij experiment 4 plaatst u canules op 2 verschillende plaatsen. De commissie gaat er van uit dat u in één rat niet op twee plaatsen canules plaatst. Dit is echter niet geheel duidelijk bij 3.4.2 en in DAP3. U dient dit overal helder en op dezelfde manier weer te geven of u twee gebieden in één dier meet, dan wel één gebied per dier.
Antwoord: Rats will undergo a surgery to unilaterally place guide cannulas into the striatum and LHb in the same animal. In other groups of animals, we will measure neurotransmitters in the medial PFC (mPFC) and LHb by unilaterally implanting two guide cannulas in the regions mentioned.
The rats thus get two cannulas in their brain. This 1. saves animals, 2. does not cause more suffering for animals since it will be done during one surgery, and 3. Allows investigation of correlations between neurotransmission changes in the two areas.

– Bij 3.4.3 geeft u weliswaar milestones aan, maar er is geen duidelijke fasering in de tijd en er zijn geen go / no go momenten benoemd. De commissie denkt echter dat het bijvoorbeeld voor de hand ligt dat experiment 1 eerst wordt uitgevoerd en dat bijvoorbeeld experiment 5 afhankelijk is van de uitkomsten van experimenten 2 en 3. U dient helder de volgorde van de testen in de tijd en de tussenliggende keuzemomenten en go/no go beslissingen aan te geven.
Antwoord: Our project has 5 milestones, namely: pilot of DAP1, main experiments of DAP1 (experiment 1 and 5), main experiments of DAP2 (experiment 2 and 3), pilot of DAP3 and main experiment of DAP3 (experiment 4). Across experiments 5-HTT and DAT knockout rats are used. Upon finishing the pilot of DAP1, we will reach the first decision point before executing the actual experiment described in DAP1. Firstly, this pilot will show us the optimal behavioural tasks to assess depression and mania like behaviour induced by imipramine, and whether these tests can be repeatedly administered without major repetition effects. We expect that imipramine will significantly increase mania-like symptoms and decrease depression-like symptoms in 5-HTT KO rats on at least 2 tests. We also expect validation of mania-like symptoms in DAT KO rats in at least 2 tests (Demontis; 2015, Goldberg; 2003, Frye, 2009). In case we do not find effects of imipramine on behavioural tasks, we will discuss our next course of action during an interim consultation with AWB. Furthermore, the pilot will inform us on the efficacy of our imipramine administration route. If imipramine given through the drinking water is ineffective in inducing mania, we will ask for experimental adjustments through an amendment. For instance, another option would be to administer imipramine via oral gavage (drawback: more stress). The results from pilot 0A of DAP1 will use as go/no go for experiment 1 and 5 (DAP 1). In experiment 1, we study the effect of imipramine treatment on behaviour in relation to depression and mania like disorders. In DAP2 we will address how imipramine treatment affects expression levels of glutamate

system components (experiment 2) or the electrophysiological features of neurons in different brain regions (experiment 3). DAP3 allows us to establish the effect of imipramine treatment on brain neurotransmitter level in relation to depression and mania like disorders. Prior to the main experiment, a pilot study (experiment 0B) will be executed with surplus animals to practice surgeries on these rats. Upon finishing pilot of DAP3, we can find the proper coordinates of the striatum, LHb and mPFC in the brain for the microdialysis experiment. In experiment 4 we measure the level of dopamine, serotonin (as well as their metabolites), and ii) glutamate (as well as GABA and glutamine) in different brain areas. Based on the molecular, electrophysiological and neurochemical data together, as well as data collected by other consortium members of other countries, we will decide about the best glutamatergic intervention. If there is no change in any of the glutamatergic measurements we will not conduct experiment 5. Hence, experiment 2, 3 and 4 serve as go/no go for experiment 5. In experiment 5, we will investigate the effect of imipramine plus N-acetylcysteine, LY2140023 or memantine (glutamatergic intervention) on depression- and mania-like behaviour.

References:
Demontis, Francesca, et al. "Memantine prevents "bipolar-like" behavior induced by chronic treatment with imipramine in rats." European journal of pharmacology 752 (2015): 49-54.
Goldberg, Joseph F., and Christine J. Truman. "Antidepressant inducedmania:an overview of current controversies." Bipolar Disorders 5.6 (2003): 407-420.
Frye, Mark A., et al. "Correlates of treatment-emergent mania associated with antidepressant treatment in bipolar depression." American Journal of Psychiatry 166.2 (2009): 164-172.

Description of Animal Procedures:
*DAP1
– 2B: U geeft bij 2B nadere uitleg over het gebruik van mannen en vrouwen.
Zoals hierboven reeds genoemd, dient u dit ook in het projectgedeelte bij de strategie (3.4) te doen. Ook kan de commissie zich voorstellen dat na het pilot experiment duidelijk wordt of beide geslachten afzonderlijk bestudeerd moeten worden of dat gemengde groepen gebruiken tot de mogelijkheid behoort. Een alternatieve benadering zou kunnen zijn eerst in één geslacht de experimenten uit te voeren en alleen daar waar belangrijke bevindingen zijn, deze ook uit te voeren met dieren van het andere geslacht.
Antwoord: The explanation about the reason of using both sexes has been set out in our answer to question 3. We added the information to the strategy (3.4) part.
For the main experiment we will use both sexes because we would like to compare the results for male and female to find out what is effect of imipramine on both sexes. We do not think it is efficient to test first one sex, and then the other, because that will take twice as many animals. We breed the animals ourselves, and thus animals of both sexes are being born. It is most efficient to use the males and females simultaneously, otherwise extra breeding is needed.

– 2B: U dient de aantallen hier, maar ook in de andere DAPs en de NTS goed na te rekenen en met elkaar in overeenstemming te brengen.
Antwoord: In DAP1 we will use 308 (Pilot A0= 44, Exp1= 88, Exp 5= 176) rats
In DAP 2 we will use 384 (EXP 2= 128, Exp 3= 256) rats

In DAP 3 we will use 180 (Pilot 0B= 20; Exp 4= 160) rats

The total number of animals for the entire project is 872.
All calculations were correct except the ones mentioned bellow:
- *In DAP1 in the following table, the number of 5-HTT KO/ WT changes from 66 to 88.*

Species	Origin	Maximum number of animals	Life stage
5-HTT KO	Own breeding	88	Adult (> PND 70)
5-HTT WT	Own breeding	88	Adult (> PND 70)
DAT KO	Own breeding	66	Adult (> PND 70)
DAT WT	Own breeding	66	Adult (> PND 70)

- in NTS total number of animals changed from 828 to 872.

– Volgens de commissie is er bij deze experimenten geen sprake van pijn en dient vraag H met nee te worden beantwoord. De forced swim test dient bij 2I genoemd te worden (als u die al gaat uitvoeren).
Antwoord: Regarding question H, we changed the answer to No.
Regarding question 2I, we removed the sentences from part H and added them to part I:

The rats are subjected to the forced swim test. In this test the water will be set at 24 ^{0}C to reduce stress, and the animals will be immediately removed from the water when their head is below the water surface. So acting immediately can reduce the stress and discomfort of animals during the test. Hence, this procedure does not exhaust the animals.

Niet-technische samenvatting:
– 3.3: Volgens de commissie moet hier 872 ratten staan; graag project en NTS met elkaar in overeenstemming brengen.
Antwoord: We calculated the number of rats again and 872 is right number. We have replaced it in NTS:
"In dit project maken we gebruik van mannelijke en vrouwelijke ratten. We zullen maximaal 872 ratten gebruiken".

– Bij 3.4 dient bijkomen uit anesthesie genoemd te worden. Een deel van de dieren wordt geoperereerd.
Antwoord: We have mentioned the recovery from anesthesia.

– Bij 3.5 graag exactere percentages aangeven.
Antwoord: DAP1: in total we will use 308 animals and the discomfort for all of them will be moderate.
DAP2: in total we will use 384 animals and the discomfort for all of them will be mild.
DAP3: in total we will use 180 animals and the discomfort for all of them will be moderate.
Moderate: 308 + 180 = 488 rats= 56%
Mild: 384 rats = 44%
"Mild voor 44 % van de dieren, en matig voor 56 % van de dieren".

- De antwoorden hebben geleid tot aanpassing van de aanvraag

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

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er is geen betrokkenheid van DEC-leden bij deze projectaanvraag, waardoor onafhankelijkheid en onpartijdigheid zijn gewaarborgd.

C. Beoordeling (inhoud)

1. Deze basaal wetenschappelijke aanvraag richt zich op het achterhalen van de mechanismen die ten grondslag liggen aan de switch van depressie naar manie bij patiënten met bipolaire stoornis. Bipolaire stoornis (BD) heeft een sterke erfelijke component. De aandoening is geassocieerd met de verminderd actieve variant van de serotonine transporter (5-HTTLPR s-allel), en met polymorfisme van de dopamine transporter (DAT). Voor beide genetische polymorfismen bestaan ratmodellen, die gebruikt zullen worden voor dit onderzoek. De 5-HTT KO ratten vertonen zowel depressie-achtig gedrag als manie-achtig gedrag. DAT KO ratten vertonen manie-achtig gedrag. Het onderzoek richt zich op glutamaat, omdat bekend is dat bipolaire stoornis gepaard gaat met verhoogde glutamaterge activiteit. Het antidepressivum imipramine (een tricyclisch antidepressivum) wordt gebruikt voor de behandeling van depressie, maar kan soms bij mensen met een bipolaire stoornis een manische episode veroorzaken. Het effect van imipramine-blootstelling wordt in beide ratmodellen onderzocht, waarbij zowel naar effecten op gedrag wordt gekeken (*in vivo*) als naar veranderingen in moleculaire markers, in concentraties van neurotransmitters en in exciteerbaarheid van hersencellen in bepaalde hersengebieden (*ex vivo*). Ten slotte wordt, bij wijze van proof of concept, onderzocht of een combinatiebehandeling van imipramine met medicijnen die glutamaat-signaling verminderen het manie-achtige gedrag kan voorkomen of afzwakken. De commissie constateert op grond daarvan dat deze aanvraag een concrete, goed afgebakende doelstelling heeft en getypeerd kan worden als een project. De opzet komt het best overeen met voorbeeld 1 uit de 'Handreiking invulling definitie project'. De verschillende subdoelen zijn noodzakelijk om de doelstelling te behalen. Het is niet mogelijk om de individuele doelen te toetsen, omdat er sprake is van onderlinge afhankelijkheid en zij tezamen een geïntegreerd begrip van de mechanismen die betrokken zijn bij de switch naar manie geven. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. De aanvrager heeft, zowel binnen de doelstellingen en bijlagen dierproeven, als tussen de doelstellingen, beschreven op basis van welke criteria zij zal besluiten het project voort te zetten. De DEC is er daardoor van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. Gezien bovenstaande is de DEC van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.

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

3. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.

Belangen en waarden

4. Het directe doel van het project is het onderzoeken van de neurobiologische mechanismen die ten grondslag liggen aan de omslag naar manie tijdens imipramine behandeling voor depressieve episodes van bipolaire stoornis in ratmodellen voor deze ziekte. Het uiteindelijke doel is bij te dragen aan de ontwikkeling van een adequatere behandeling van depressieve episodes die optreden bij mensen met bipolaire stoornis, zonder daarbij een manische episode op te wekken. In dit project wordt het effect van behandeling met imipramine op rattenhersenen en –gedrag bestudeerd, en wanneer het leidt tot manie-achtig gedrag dan wordt getracht dit gedrag met medicijnen die glutamaat-signaling verminderen te reduceren. Op die manier wordt een indicatie gekregen van de mogelijkheid om een combinatiebehandeling te ontwikkelen voor BD-patiënten die minder risico geeft op het induceren van een manische episode. Er is daarom binnen deze aanvraag wel een reële maar geen directe relatie tussen het doel van deze projectaanvraag en het uiteindelijke doel. De aanvrager heeft duidelijk gemaakt dat de kennis van de mechanismen die de omslag naar manie veroorzaken tijdens imipramine-behandeling nog zeer beperkt is, dat deze kennis nodig is voor de ontwikkeling van nieuwe behandelingen, en dat er behoefte is aan nieuwe behandelingen. Naar de mening van de DEC is het doel van deze projectaanvraag daarom gerechtvaardigd binnen de context van het onderzoeksveld.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.
   Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast (zie C11 en C12). De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.
   Voor de onderzoekers geldt dat het publiceren van belangrijke nieuwe wetenschappelijke inzichten resulteert in een goede wetenschappelijke reputatie, hetgeen vaak de sleutel is voor het verkrijgen van nieuwe onderzoeksmiddelen en mogelijkheden. Dit kan door de onderzoeker en de betrokken organisaties zelf van belang geacht worden, maar dient naar de mening van de DEC geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren. Het gaat uiteindelijk om de vraag of dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis).
   Voor patiënten is dit onderzoek indirect en op de lange termijn van belang, omdat het kan leiden tot de ontwikkeling van betere diagnostiek en/of effectievere behandelingen voor bipolaire stoornis. Behandeling van deze aandoening is lastig, omdat behandeling van een depressieve periode met antidepressiva kan omslaan in een manie en vice versa. De resultaten van dit project geven meer inzicht in de mechanismen die deze omslag veroorzaken, en kunnen bijdragen aan de ontwikkeling van een behandeling van depressieve periodes die minder risico geeft op deze bijwerking. Dit kan er toe leiden dat de patiënt een betere kwaliteit van leven heeft. Kunnen beschikken over adequate behandelingen voor ernstige psychiatrische aandoeningen, zoals bipolaire stoornis, is van groot belang voor de samenleving.

6. De aanvrager maakt geen melding van onbedoelde nadelige effecten op het milieu. Er is geen aanleiding voor de DEC om te verwachten dat die er zullen zijn.

Proefopzet en haalbaarheid

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd. De aanvrager heeft zeer veel ervaring met gedragsonderzoek in

ratmodellen voor depressie en andere psychiatrische aandoeningen. Het onderzoek heeft geresulteerd in tal van publicaties in goede wetenschappelijke tijdschriften. De commissie is daarom overtuigd van de kwaliteit van het werk van de aanvrager. De aanvrager beschikt over voldoende kennis en kunde, onder andere op grond van een artikel 9 kwalificatie, om te kunnen voldoen aan alle zorgvuldigheidseisen omtrent het verrichten van dierproeven.

8. De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet en uitkomstparameters sluiten hier merendeels logisch bij aan (zie C1 en C4). De commissie heeft bij de aanvragers haar twijfels geuit over de bruikbaarheid van de forced swim test (FST) voor het meten van depressie. Zelfs binnen deze leerstoelgroep verschillen kennelijk de meningen hierover (zie de correspondentie in het advies van de DEC over AVD1030020187064: de onderzoekers schrapten de test vanwege "beperkte betrouwbaarheid"). De aanvragers houden echter vast aan het gebruik van deze test. De commissie constateert dat de waarde van deze test als maat voor depressie in het onderzoeksveld (en nota bene ook binnen de eigen leerstoelgroep) in twijfel wordt getrokken. Verder is de commissie van mening dat deze test naast de 'batterij' van andere testen die de aanvrager ter beschikking heeft, weinig toegevoegde waarde heeft in dit onderzoek. Tot slot is het voor de commissie van belang dat ook de mate van ongerief die deze test met zich meebrengt voor discussie vatbaar is (Bijlage VIII van de Richtlijn spreekt over "ernstig", de aanvrager houdt het op "matig"). Het uitvoeren van een test met mogelijk ernstig ongerief (al betwijfelt de commissie of dat bij de in dit project voorgestelde werkwijze het geval zou zijn), terwijl de waarde ervan twijfelachtig is en de test ook niet onmisbaar lijkt voor het behalen van de doelstellingen van dit project, lijkt de commissie moeilijk te verdedigen. Al met al is de commissie dan ook van mening dat er geen goede redenen zijn om de aanvrager vergunning te verlenen voor het uitvoeren van deze test in het kader van dit project. Deze groep heeft veel ervaring in dit onderzoeksveld en met de voorgestelde dierproeven. De DEC is dan ook van oordeel dat het project verder goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project.

Welzijn dieren

9. Er is sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
☐ Bedreigde diersoort(en) (10e lid 4)
☐ Niet-menselijke primaten (10e)
☐ Dieren in/uit het wild (10f)
☐ Niet gefokt voor dierproeven (11, bijlage I richtlijn)
☐ Zwerfdieren (10h)
☐ Hergebruik (1e lid 2)
☐ Locatie: buiten instelling vergunninghouder (10g)
☐ Geen toepassing verdoving/pijnbestrijding (13)
☒ Dodingsmethode niet volgens bijlage IV richtlijn (13c lid 3)
De aanvrager heeft als volgt onderbouwd dat dit noodzakelijk is: In experiment 2 en 3 zullen de ratten worden gedecapiteerd zonder anesthesie voor moleculaire studies en elektrofysiologische metingen. De aanvrager stelt geen anesthesie te gebruiken, omdat dit waarschijnlijk interfereert met de moleculaire en elektrofysiologische metingen. Anesthetica hebben een effect op glutamaat neurotransmissie [*Rammes, Gerhard, et al. "Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B subunit expression." Neuropharmacology 56.3 (2009): 626-636.*] De DEC is het eens met deze onderbouwing. De onderzoekers hebben veel ervaring met deze dodingsmethode waardoor deze snel wordt uitgevoerd en niet veel stress oplevert voor de

dieren.

10. De huisvesting en verzorging van de dieren zijn voor het grootste deel van de studie conform de eisen in bijlage III van richtlijn 2010/63/EU. De meeste dieren zullen echter wel tweemaal drie dagen individueel gehuisvest worden in het kader van een gedragsexperiment. De DEC is van mening dat dit gedragsexperiment niet zonder individuele huisvesting uitgevoerd kan worden.

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd als licht voor 44% van de dieren en matig voor de rest van de dieren. Het ongerief wordt hoofdzakelijk veroorzaakt door de batterij gedragsexperimenten waarmee depressief gedrag en manisch gedrag gemeten kan worden, en door de operatie waarbij twee canules geplaatst worden. Volgens bijlage VIII van richtlijn 2010/63/EU is de forced swim test een voorbeeld van een procedure die ernstig ongerief veroorzaakt. Daar de commissie van mening is dat er geen goede redenen zijn om de aanvrager vergunning te verlenen voor het uitvoeren van deze test in het kader van dit project (zie C8), is een discussie over de mate van ongerief in dit advies echter overbodig. Het cumulatief ongerief als gevolg van de resterende dierproeven is ten hoogste matig.

12. De integriteit van dieren wordt aangetast door het instrumentele gebruik van de dieren dat inherent is aan het doen van dierproeven. De helft van de dieren in dit project heeft een erfelijke afwijking waardoor bepaalde neurotransmitters in de hersenen anders werken. Bij een aantal dieren worden hersencanules geplaatst om de concentratie van bepaalde neurotransmitters te kunnen bepalen. Het dier wordt hierdoor gehinderd in zijn normale gedrag.

13. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op het experiment. Het percentage geopereerde dieren dat naar verwachting een humaan eindpunt zal bereiken is op basis van ervaring met deze operatie ingeschat op 20%. Voor de overige dieren is het percentage dieren dat een humaan eindpunt zal bereiken naar verwachting zeer laag en op basis van eigen ervaring en gegevens uit de wetenschappelijke literatuur ingeschat. De commissie is het eens met deze inschattingen en de gehanteerde humane eindpunten.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. Om moleculaire metingen te kunnen relateren aan gedrag zijn experimenten met dieren noodzakelijk.

15. Het maximale aantal te gebruiken dieren is realistisch ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet en de looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog een wetenschappelijk betrouwbaar resultaat kan worden verkregen. Door de longitudinale opzet van de gedragsexperimenten, waarbij elk dier zijn eigen controle is, zijn er in totaal minder dieren nodig. Door de stapsgewijze aanpak waarin de resultaten uit eerdere proeven worden gebruikt voor het design van vervolgexperimenten wordt onnodig gebruik van proefdieren voorkomen.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. Imipramine wordt via het drinkwater gegeven, zodat er geen injecties nodig zijn. De onderzoekers zullen verdoving toepassen voor de handelingen waarvoor dit vereist is. De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo

humaan mogelijk worden uitgevoerd.

17. Het betreft geen wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Dieren van beide geslachten zullen in gelijke mate ingezet worden. Bipolaire stoornis komt voor bij mannen en vrouwen, maar er zijn kwalitatieve verschillen in het ziektebeeld. Daarom willen de onderzoekers alle experimenten zowel met mannelijke als met vrouwelijke dieren uitvoeren. De commissie heeft gevraagd het gebruik van zowel mannelijke als vrouwelijke dieren beter te onderbouwen, en uit te leggen waarom er geen gebruik kan worden gemaakt van gemengde groepen. De onderzoekers beargumenteren dat het mechanisme achter de sexe-verschillen in het ziektebeeld tussen mannen en vrouwen niet bekend is, waardoor dit misschien de uitslagen van de experimenten zou kunnen verstoren wanneer gemengde groepen worden bestudeerd. Hierdoor zouden experimenten herhaald moeten worden met uitsluitend mannen en/of uitsluitend vrouwen, waardoor er meer dieren nodig zijn. Ook kunnen de resultaten van experimenten verschillen tussen de geslachten, waardoor het niet mogelijk is om de proeven eerst met ene geslacht uit te voeren en relevante experimenten in het andere geslacht te herhalen. Voorts zal het gebruik van zowel mannelijke als vrouwelijke dieren uit eigen fok tot geringere fokoverschotten leiden. De commissie is van mening dat de onderzoekers afdoende hebben onderbouwd waarom het noodzakelijk is om alle experimenten zowel met mannelijke als met vrouwelijke dieren uit te voeren, en dat het gebruik van gemengde groepen niet wenselijk is en ook niet leidt tot het gebruik van minder dieren.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om verschillende weefsels te kunnen onderzoeken voor het beantwoorden van bepaalde onderzoeksvragen. De gebruikte dodingsmethode staat vermeld in bijlage IV van richtlijn 2010/63/EU, maar mag alleen gebruikt worden indien de andere methoden niet mogelijk zijn. Voor het behalen van de doelstelling is het gebruik van deze dodingsmethode noodzakelijk (zie onderdeel C9 van dit advies).

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

NTS

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

D. Ethische afweging

1. Rechtvaardigt het belang van meer inzicht in de neurobiologische mechanismen die ten grondslag liggen aan de omslag naar manie tijdens imipramine behandeling voor depressieve episodes van bipolaire stoornis in ratmodellen voor deze ziekte, het ongerief dat de dieren wordt aangedaan, en is aan alle zorgvuldigheidseisen (3V's) voldaan?

2. Er vindt een lichte of ten hoogste matige (voor 56% van de dieren) aantasting van het welzijn en een aantasting van de integriteit van de proefdieren plaats. De doelstellingen kunnen niet zonder dieren behaald worden. De onderzoekers doen al het mogelijke om het lijden van de dieren en het aantal dieren te beperken (beschreven in C9 tot C20).

Voor patiënten is dit onderzoek op de lange termijn van belang, omdat het kan bijdragen aan een verbetering van hun geestelijke gezondheid en kwaliteit van leven. De DEC kent daar veel gewicht aan toe om de volgende redenen. Bipolaire stoornis is immers een psychiatrische ziekte die zich op jongvolwassen leeftijd openbaart. De impact van de ziekte op patiënten en hun directe omgeving is groot: ongeveer één op de zes patiënten pleegt zelfmoord. Op dit moment zijn er weinig medicijnen beschikbaar voor een effectieve behandeling. Het blijkt met name lastig om depressieve episodes te behandelen, zonder daardoor een manische episode te induceren. De resultaten van dit project zullen bijdragen aan meer inzicht in de neurobiologische mechanismen die betrokken zijn bij de omslag naar manie tijdens de behandeling van een depressie, en aan de ontwikkeling van een nieuwe (combinatie)therapie met minder risico op het induceren van een manie. Het is aannemelijk dat de doelstellingen op termijn behaald zullen worden. De commissie acht het ontwikkelen van een nieuwe (combinatie)therapie voor BD van substantieel belang.
Meer inzicht in neurobiologische mechanismen is ook voor andere hersenonderzoekers van belang.

3. De DEC is overtuigd van het belang van de doelstellingen: het onderzoeken van de neurobiologische mechanismen die ten grondslag liggen aan de omslag naar manie tijdens imipramine behandeling voor depressieve episodes van bipolaire stoornis in ratmodellen voor deze ziekte. Het uiteindelijke doel daarvan is bij te dragen aan de ontwikkeling van een adequatere behandeling van depressieve episodes die optreden bij mensen met bipolaire stoornis, zonder daarbij een manische episode op te wekken. De DEC is van mening dat de belangen van de patiënten voldoende zwaar wegen om het schaden van de belangen van de proefdieren (om gevrijwaard te blijven van een aantasting van hun welzijn en integriteit) te rechtvaardigen. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De DEC is van mening dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat zij zal kunnen voorkomen dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.
De DEC is van oordeel dat het hier boven geschetste belang de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

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

   ☒ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
   - o 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.
   - o Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
   - X Overige door de DEC aan de uitvoering verbonden voorwaarden: de aanvrager dient het gebruik van de Forced Swim Test in dit onderzoek naar het oordeel van de DEC achterwege te laten (zie C8).

☐ De DEC adviseert de vergunning niet te verlenen vanwege:

- **De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:...**
- **De volgende doorslaggevende ethische bezwaren:...**
- **De volgende tekortkomingen in de aanvraag:...**

2. Het uitgebrachte advies is gebaseerd op consensus.

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

Van: Info-zbo <info@zbo-ccd.nl>
Verzonden: Vrijdag 14 juni 2019 17:25
Aan: Postbus instantie voor dierenwelzijn
CC: [redacted]
Onderwerp: Vervolg aanvraag
AVD1030020197744

Geachte [redacted]
Op 4 april 2019 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Unveiling the mechanism(s) underlying the switch to mania during antidepressant treatment: The role of glutamate" met aanvraagnummer AVD1030020197744. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

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

1) In bijlage 3.4.4.1 wordt beschreven dat de dieren worden gedood, maar de reden waarom is niet aangegeven. Kunt u de reden van doden van de dieren toevoegen bij vraag L?
2) In bijlage 3.4.4.1 beschrijft u een drop-out van 10% vanwege het longitudinale design van de studie. Hoewel wij begrijpen dat u in een longitudinale studie rekening houdt met uitval, willen wij u vragen dit toch nader te beschrijven. Kunt u nader beschrijven waarom u hier kiest voor 10% drop-out en wat de oorzaken van de uitval zijn?

Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

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

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

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

Met vriendelijke groet,

[redacted]

Namens:
Centrale Commissie Dierproeven

www.centralecommissiedierproeven.nl

.....
Postbus 93118 | 2509 AC | Den Haag

Dear CCD

Thank you for your questions regarding DEC 2019-005 entitled: Unveiling the mechanism(s) underlying the switch to mania during antidepressant treatment: The role of glutamate. Our answers to these questions can be found below.

Question 1

Appendix 3.4.4.1 describes that the animals are killed, but the reason why is not specified. Can you add the reason for killing the animals in question L?

The aim of using animals in the experiments 0A, 1 and 5 is studying the different behaviors of rats related to depression and mania due to pharmacological treatment. Because the animals received pharmacological treatment, they are not suitable to function as surplus animals in experiments of other researchers. Therefore, we suggest to sacrifice the animals 24 hours after the last behavioral test by CO2 (method that is typically used if no biomaterials of the animals are needed). [*This text has been added to the DAP*]

Question 2

In appendix 3.4.4.1 you describe a drop-out of 10% due to the longitudinal design of the study. Although we understand that you take failure into account in a longitudinal study, we would ask you to describe this in more detail. Can you describe in more detail why you opt for a 10% dropout here and what the causes of the failure are?

A power analysis based on depression-related measures in 5-HTT KO rats (Olivier, 2008) revealed an effect size of 0.62, making that at α=0.05 and 1-β=0.8, an N=10 is really needed for these behavioral studies. We increase N=10 to N=11 to cover 10% drop out because our experiment will last for 10 weeks and it is quite long time for testing. There is for instance the possibility that one of the animals loses weight and reaches the humane end point f.i. because water consumptive drops due to changing its taste with imipramine. This drop out rate is based on our experience.

Centrale Commissie Dierproeven

> Retouradres Postbus 93118 2509 AC Den Haag

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

Centrale Commissie Dierproeven
Postbus 93118
2509 AC Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD1030020197744
Bijlagen
1

Datum 9 juli 2019
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 4 april 2019 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Unveiling the mechanism(s) underlying the switch to mania during antidepressant treatment: The role of glutamate" met aanvraagnummer AVD1030020197744. Wij hebben uw aanvraag beoordeeld.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a lid 1 van de Wet op de dierproeven (hierna: de wet).

U kunt met uw project starten. De vergunning wordt afgegeven van 9 juli 2019 tot en met 31 mei 2023.
De onderbouwing van deze beslissing vindt u onder 'Overwegingen'.

Voorwaarden
Aan de vergunning hebben wij voorwaarde(n) verbonden op grond van artikel 10a1 lid 2 van de wet. Deze voorwaarde(n) vindt u in het deel 'Projectvergunning' van dit besluit. Onder 'Overwegingen' lichten wij toe waarom wij deze voorwaarde(n) aan de vergunning verbinden.

Datum:
9 juli 2019
Aanvraagnummer:
AVD1030020197744

Procedure

Advies dierexperimentencommissie
Wij hebben advies gevraagd bij de Dierexperimentencommissie (DEC) RU DEC. Dit advies is ontvangen op 15 mei 2019. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a lid 3 van de wet. Het advies van de DEC is betrokken bij de behandeling van uw aanvraag.

Nadere vragen aanvrager
Op 14 juni 2019 hebben wij u om aanvullingen gevraagd. U heeft antwoord gegeven. De aanvullingen hadden betrekking op uitleg over de reden van doden van de dieren, en nadere onderbouwing van de te verwachten uitval van dieren. Uw antwoord is betrokken bij de behandeling van uw aanvraag.

Overwegingen

Alle hierboven genoemde stukken liggen ten grondslag aan ons besluit.

Wij kunnen ons vinden in de inhoud van het advies van de DEC, inclusief de daaraan ten grondslag liggende motivering.

Voorwaarden
Wij hebben de volgende bijzondere voorwaarde opgelegd:
De Forced Swim Test (FST) mag niet worden toegepast in dit onderzoek.

Zoals ook door de DEC geconstateerd, wordt de waarde van de FST als maat voor depressie in het onderzoeksveld in twijfel getrokken. Daarnaast is de CCD het eens met de DEC dat de FST naast de andere testen die u ter beschikking heeft, weinig toegevoegde waarde heeft in dit onderzoek omdat er reeds andere gedragstesten gebruikt worden die een indicatie van depressief gedrag kunnen geven, zoals de anhedonia/motivation task en de sleep/wake patterns.
Ook lijkt de test niet onmisbaar voor het behalen van de doelstellingen van dit project.
Tot slot is het van belang dat ook de mate van ongerief die deze test met zich meebrengt voor discussie vatbaar is. Bijlage VIII van de Richtlijn spreekt over "ernstig ongerief", terwijl u van mening bent dat er sprake is van "matig ongerief". Er wordt een test uitgevoerd met mogelijk ernstig ongerief, terwijl de waarde ervan als maat voor depressie in twijfel wordt getrokken.
Concluderend hebben de bovengenoemde redenen tot het besluit geleid dat deze test niet vergund zal worden.

De CCD is het eens met de DEC dat het project verder goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project.

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 93118, 2509 AC 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.

Datum:
9 juli 2019
Aanvraagnummer:
AVD1030020197744

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

Centrale Commissie Dierproeven
namens deze:

i.o.
[redacted]
Algemeen Secretaris

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

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

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

deze projectvergunning voor het tijdvak 9 juli 2019 tot en met 31 mei 2023, voor het project "Unveiling the mechanism(s) underlying the switch to mania during antidepressant treatment: The role of glutamate" met aanvraagnummer AVD1030020197744, volgens advies van Dierexperimentencommissie RU DEC.
De functie van de verantwoordelijk onderzoeker is [redacted].
Het besluit is gebaseerd op de volgende (aangepaste) stukken:

1 een aanvraagformulier projectvergunning dierproeven, zoals ontvangen op 4 april 2019
2 de bij het aanvraagformulier behorende bijlagen:
  a Projectvoorstel, zoals ontvangen op 15 mei 2019;
  b Bijlagen dierproeven
    - 3.4.4.1 Behaviour, zoals ontvangen op 26 juni 2019;
    - 3.4.4.2 Ex vivo measurements, zoals ontvangen op 26 juni 2019;
    - 3.4.4.3 Surgery, zoals ontvangen op 26 juni 2019;
  c Niet-technische Samenvatting van het project, zoals ontvangen op 15 mei 2019;
  d Advies van Dierexperimentencommissie zoals ontvangen op 15 mei 2019
  e De aanvullingen op uw aanvraag, ontvangen op 26 juni 2019.

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst
3.4.4.1 Behaviour			
	Ratten (Rattus norvegicus) / 5-HTT KO en 5-HTT WT	308	100,0% Matig
3.4.4.2 Ex vivo measurements			
	Ratten (Rattus norvegicus) / 5-HTT KO en WT, DAT KO en WT	384	100,0% Licht
3.4.4.3 Surgery			
	Ratten (Rattus norvegicus) / 5-HTT KO en WT, DAT KO en WT	180	100,0% Matig

Aanvraagnummer:
AVD1030020197744

Voorwaarden

Bijzondere voorwaarden

De Forced Swim Test mag niet worden toegepast in dit onderzoek.

Ter informatie
Onderstaande informatie is opgenomen op grond van artikel 1d lid 4, artikel 10a1 lid 2, artikel 10 lid 2 en/of artikel 10a3 van de wet.

- Go/ no go momenten worden voor aanvang van elk experiment afgestemd met de IvD.
- Het is verboden 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.
- Het is verboden dierproeven te verrichten voor een doel waarvan het belang niet opweegt tegen het ongerief dat aan het proefdier wordt berokkend.
- Overige wettelijke bepalingen blijven van kracht.

Centrale Commissie Dierproeven

Aanvraagnummer:
AVD1030020197744

Weergave wet- en regelgeving

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

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.