	Inventaris Wob-verzoek W23-03									
		wordt verstrekt				weigeringsgronden				
nr.	document NTS 202216352	reeds openbaar	niet	geheel	deels	5.1, lid 1c	5.1, lid 2e	5.1, lid 2f	5.1, lid 2h	5.2, lid 1
1	Aanvraag projectvergunning, d.d. 04-08-2022				x		x		x	
2	Projectvoorstel bij aanvraag				x	x			x	
3	Bijlage dierproeven_1 bij aanvraag				x	x			x	
4	Bijlage dierproeven_2 bij aanvraag				x	x			x	
5	Bijlage dierproeven_3 bij aanvraag				x	x			x	
6	Bijlage dierproeven_4 bij aanvraag				x	x			x	
7	NTS bij de aanvraag				x	x			x	
8	E-mail aan DEC om advies aanvraag projectvergunning, d.d. 22-08-2022				x				x	
9	DEC-advies, d.d. 13-02-2023				x	x	x		x	
10	Aanvraag na DEC advies				x		x		x	
11	Projectvoorstel na DEC advies				x	x			x	
12	Bijlage_1 dierproeven na DEC advies				x	x			x	
13	Bijlage_2 dierproeven na DEC advies				x	x			x	
14	Bijlage_3 dierproeven na DEC advies				x	x			x	
15	NTS a DEC advies				x				x	
16	AdviesnotaCCD, d.d. 15-02-2023_met opmerkingen				x	x	x		x	x
17	AdviesnotaCCD, d.d. 01-03-2023				x	x	x		x	x
18	E-mail CCD aan vergunninghouder over aanvraag, d.d. 01-03-2023				x		x		x	
19	Reactie na vragen CCD				x				x	
20	Projectvoorstel na CCD vragen				x	x			x	
21	Bijlage_1 dierproeven na CCD vragen				x	x			x	
22	Bijlage_2 dierproeven na CCD vragen				x	x			x	
23	Bijlage_3 dierproeven na CCD vragen				x	x			x	
24	NTS na CCD vragen en definitieve versie			x						
25	AdviesnotaCCD, d.d. 13-03-2023				x	x	x		x	x
26	Beschikking, d.d. 13-03-2023				x		x		x	
27	E-mail CCD aan DEC over aanvraag projectvergunning, d.d. 17-03-2023				x		x		x	

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 neem telefonisch contact op. (0900-2800028).

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* 5.1 lid2h

☐ Nee > *U kunt geen aanvraag doen*

1.2 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 1.3

☐ Wijziging > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.1

☐ Melding > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.2

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

Naam instelling of organisatie	5.1 lid2h			
Titel, voorletters en achternaam van de portefeuillehouder	Titel	Voorletters	Achternaam	☐ Dhr. ☐ Mw
	5.1 lid2e			
E-mailadres contactpersoon	5.1 lid2h			
Titel, voorletters en achternaam van de diens gemachtigde (indien van toepassing)	Titel	Voorletters	Achternaam	☐ Dhr. ☐ Mw
E-mailadres gemachtigde				

Vul de gegevens van het postadres in.

Straat en huisnummer	5.1 lid2h
Postcode en plaats	
Postbus, postcode en plaats	

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	5.1 lid2e	☐ Dhr. ☐ Mw.
Functie	5.1 lid2e	
Afdeling	5.1 lid2h	
Telefoonnummer	5.1 lid2e	

	E-mailadres	5.1 lid2e	
1.5 *(Indien van toepassing)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.	(Titel) Naam en voorletters	5.1 lid2e	☐ Dhr. ☐ Mw.
	Functie	5.1 lid2e	
	Afdeling	5.1 lid2h	
	Telefoonnummer	5.1 lid2e	
	E-mailadres	5.1 lid2e	
1.6 *(Indien van toepassing)* 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		
1.7 *(Optioneel)* Vul hier de gegevens in van de Instantie voor Dierenwelzijn	Telefoonnummer		
	E-mailadres		
1.8 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 3

☐ Ja > Geef hier onder kort de wijziging en de onderbouwing daarvan weer. Geef in de originele formulieren (niet-technische samenvatting, projectvoorstel en bijlage dierproeven) duidelijk aan (bij voorbeeld in een andere kleur) waar de projectaanvraag wijzigt. Ga daarna verder met vraag 6.

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	1 - 11 - 2022	
	Einddatum (t/m)	31 - 10 - 2027	
3.2 Wat is de titel van het project?	Mechanisms underlying the pathophysiology of Angelman Syndrome		
3.3 Wat is de titel van de niet-technische samenvatting?	Mechanismen die ten grondslag liggen aan Angelman Syndroom		
3.4 Wat is de naam van de Dierexperimentencommissie (DEC) van voorkeur?	Naam DEC	5.1 lid2h	
	Postadres		
	E-mailadres		

4 Factuurgegevens

4.1 (indien factuuradres afwijkt van de gegevens uit vraag 1.3) Vul de gegevens van het factuuradres in.

Naam:	Afdeling:	
Straat:		Huisnummer:
Postcode:	Plaats:	
Postbus:	Postcode:	Plaats:
E-mail:		

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

Ordernummer:

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel — Aantal bijlage(n) dierproeven 4

☒ 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 de Centrale Commissie Dierproeven (voor adresgegevens zie website)

Ondertekening door de portefeuillehouder namens de instellingsvergunninghouder of gemachtigde (zie 1.8). 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 C 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	5.1 lid2e
Functie	Gemandat
Plaats	5.1 lid2h
Datum	04 - 08
Handtekening	

5.1 lid2e

Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	Provide the title of the project.	Mechanisms underlying the pathophysiology of Angelman Syndrome

2 Categories

2.1	Please tick each of the following boxes that applies to your project. Let op! De verplichte bijlagen verschillen per categorie. Op hetInvloket.nl leest u meer informatie over de verplichte bijlagen per	☐ Basic research ☒ Translational or applied research ☐ Regulatory use or routine production ☐ Research into environmental protection in the interest of human or animal ☐ 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 2.1.

Angelman syndrome (AS) is a severe neurodevelopmental disorder affecting approximately 1:20,000 births resulting in severe developmental delay (max. developmental age is ±2 years), intellectual disability, motor dysfunction, behavioural abnormalities including autism and impaired sleep cycle, and absence of speech. A large number of patients (80%) also suffer from epilepsy.

To understand more about the basic biology underlying the pathophysiology of AS, in the last 5 years we have carried out work (PLA AVD5.1 lid2h) in which we aimed to identify functional domains of UBE3A, identify its target proteins and understand its role in neuronal function and brain development. To this end we published work in which we showed the importance of correct localisation of the majority of the UBE3A protein to the nucleus for it to be able to fulfil its function, a property of UBE3A that is shared between mice and humans [1][2]. With this knowledge in mind, when studying a cohort of Angelman individuals carrying a single amino acid change in the protein, we determined that the majority of mutations rendered the nuclear UBE3A protein cytosolic which was enough to cause AS [3]. To study the specific role of each of the nuclear or cytoplasmic UBE3A protein isoforms, we have successfully generated a number of mouse lines in the previous 5 years that express only the nuclear or cytoplasmic UBE3A isoforms. In the coming 5 years we hope to use these mouse models to help us understand the role of each isoform.

Being a ubiquitin ligase, one of the questions that still lingers concerns the target proteins ubiquitinated by UBE3A. A major data set was created in a collaborative effect between our lab and the pharmaceutical industry, in which targets of UBE3A were found in a large scale spatiotemporal proteomic analysis [4]. This valuable data set is publicly available.

A lot is still unknown about the function of UBE3A in different cellular compartments, and with which targets UBE3A interacts in these compartments. We also do not know what makes UBE3A so essential to brain development. Additionally, with a more severe phenotype in patients missing the entire 15q11-13 locus which contains many genes including *UBE3A*, it is important to disentangle the contribution of these genes within the AS pathophysiology. A better understanding of the fundamental biology and physiology underlying Angelman Syndrome can open up new directions for therapeutic strategies. These are important questions that we will address in the category 'Basic Research'.

The category 'Basic research' encompasses all studies concerning the pathophysiology of the absence of UBE3A and the role of neighbouring genes in the 15q11-13 locus as well as UBE3A target genes.

There are currently no effective treatments available for AS and patients need life-long care. 5.1 lid2h [7]. This was a proof-of-concept study that formed part of the foundation upon which the current clinical trial, being performed on AS individuals, was based. Our lab holds AS mouse models that are already used for drug testing on a regular basis. Importantly, our close connection to the clinic through the 5.1 lid2h enables us to translate preclinical findings of promising therapies to the clinic. Only drugs we test that look promising and safe when tested in our animal models will be allowed to proceed to clinical trials. The development of new therapeutics for AS is thus an important direction that we will address in the category 'Translational research'.

The category 'Translational research' encompasses all drug testing studies. We have developed a 5.1 lid2h that is very powerful for drug testing, and we are testing drugs on a regular basis in our mouse models. When these results look promising, we will test these drugs in the clinic. We have frequently demonstrated that we are able to translate the preclinical findings to the clinic through our 5.1 lid2h .

3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The ultimate goal of the project is to understand the role of UBE3A in brain development using mouse models and to identify a treatment for Angelman syndrome. More specifically we have the following goals:

Aim 1: Investigate the role of (nuclear) UBE3A in brain development. 5.1 lid2h

The proteasome is important for protein homeostasis as it rids the cell of aberrantly folded or excess proteins and through the proteasome, UBE3A will likely regulate the levels of various proteins. 5.1 lid1c

Aim 2: Study the role of UBE3A in the cytosol/synapse. Although we showed that UBE3A plays a critical role in the nucleus, we cannot rule out that in addition to the nuclear role, there is also an important role for UBE3A in the cytosol/synapse. 5.1 lid1c

Aim 3: Study the effect of neighbouring genes in the 15q11-13 gene cluster. Although specific mutations only affecting the UBE3A gene result in AS, the majority (80%) of the patients harbour a large deletion affecting maternal chromosome 15, encompassing the 15q11-13 region (±6Mb) which includes UBE3A. The symptoms experienced by these patients are typically more severe compared to individuals carrying mutations only affecting the maternal copy of UBE3A, in particular with respect to epilepsy and developmental age. 5.1 lid1c

Aim 4: Identify brain regions that are affected by loss of UBE3A and the phenotypes to which these brain regions contribute. 5.1 lid1c

Aim 5: 5.1 lid1c

3.2.2 Provide a justification for the project's feasibility.

In previous years, we have gained significant expertise in the field of Angelman syndrome and in particular exploring our various mouse models at the molecular, cellular, electrophysiological and behavioural levels (AVD 5.1 lid2h). Below is an indication of our field of expertise relevant to each appendix.

With 5.1 lid2h including high-profile journals, we are considered to be leaders in this field. Specifically, we have generated AS mouse models (Appendix 1), described robust phenotypes (Appendix 3) that are now used in labs all over the world, and we have significantly

contributed to our understanding of Angelman Syndrome (Appendix 2). We have gained significant expertise in treating mice with novel therapeutic treatment modalities, in particular antisense oligonucleotides (ASOs/AONs), and have contributed to the development of therapies which are now in clinical trials at the 5.1 lid2h (Appendices 2 and 3). The injection of iPSC derived cells into the brains of mice has been described in the literature (3) and is a project carried out in collaboration with other groups who have experience with culturing, differentiation and transplantation of human induced pluripotent stem cells (iPSCs) into young mice (AVD5.1 lid2h) (Appendix 4).

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

☒ No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

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

Angelman syndrome is a very severe neurodevelopmental syndrome in which the patients typically reach but do not surpass a developmental age of 16-24 months despite normal life expectancy. Hence the children need life-long care. Little is known about the underlying pathophysiology, and currently there is no effective treatment. Even anti-epileptic drugs are often not effective in these patients, and the behavioural problems, absence of a regular day-night time sleep rhythm, as well as the inability to talk, puts a great burden on the families. Therefore, drugs that alleviate (some) of these problems are very welcome. For the drugs to be most effective, we need to have a mechanistic understanding of the role of the different UBE3A isoforms in neurons and how this affects differentiation and/or function of neurons in different areas of the brain. Also, with UBE3A being so highly enriched in the nucleus and uniquely bound to the proteasome, a ubiquitous cellular system, there is also great scientific interest in what role it plays in cellular proteostasis. Ultimately a more complete understanding of UBE3A biology will result in novel therapeutic approaches.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

Laboratory animals: The goal of this application is to request the use of animals to help us understand the biology behind AS and to develop drugs for patients. The animals obviously have a negative interest, as their integrity can be affected by genetic modification in addition to applying drugs and performing surgeries and tests, all potentially harmful to the animal.The animals are eventually killed as part of the experiment. The welfare of the mice will be at most moderately affected during our experiments and it is our moral duty to ensure that the discomfort the animals endure is kept to a minimum.

Patients and parents: Patients (and indirectly their family members) with AS will hopefully profit from our experiments. Despite this clear positive interest, they may also need to provide cells (negative interest), often in the form of a vial of blood, from which iPSC derived cells will be generated. In addition, there are no guarantees that we succeed in identifying a treatment, and even if we do so they carry a significant risk when these therapies are applied to the patients. This risk is reduced but not excluded upon in vivo testing in animals.

The lab: The lab has a scientific interest in deciphering the aetiology of AS. The work described here is of scientific importance to the lab, as it enables us to gain knowledge pertaining to the design of genetic therapies and how the cells/mice respond to these treatments. This process may also generate scientific knowledge about the underlying aetiology of AS. The lab also generates its income from its publications, as they will facilitate funding of new projects. With respect to lab funding sources, 40% comes from government funding, 40% from patient organisations and 20% from industry.

5.1 lid2h

5.1 lid2h

Society: In terms of the societal impact, AS is a debilitating disorder that has a great impact on the afflicted individuals but certainly also on the care-givers. A treatment for some of these disorders may have a positive impact on the patient and thus on our society at large, but could also influence health costs.

Industry: some of the drugs will be developed together with the pharmaceutical industry. They will financially profit from our studies but it will increase the chance of developing a drug and get it approved for clinical trials.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

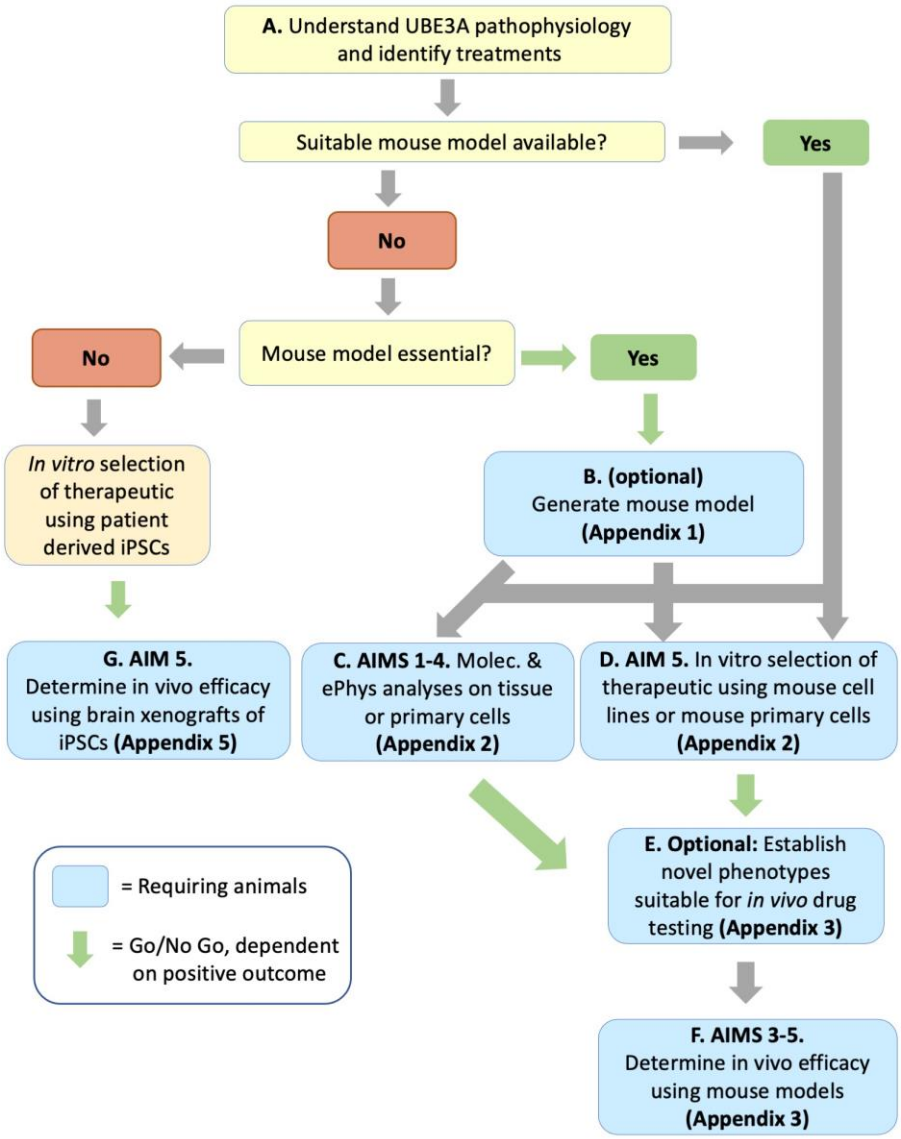


Figure 1: Overall strategy

Our research separates in two major goals: (1) Studies into the pathophysiology (and function) of UBE3A and genes in the 15q11-13 gene cluster (see 3.2.1 Aims 1-4), and (2) Drug testing to identify novel treatments **(Aim 5)**. Our lab primarily utilises molecular and cellular studies that do not require animals (including inducible pluripotent stem cells (iPSCs)). However, since UBE3A is critical for early postnatal brain development, the loss of UBE3A and the effectiveness of drugs cannot be faithfully modelled in a dish. Hence, we still need animals for a number of experiments. These types of experiments in addition to the goals to which they contribute, are summarized below:

A. Understand UBE3A pathophysiology and identify treatments: Ideally, we would carry out all experiments, in vitro, using cultured human iPSC derived cells. Unfortunately, cellular studies cannot provide us with all the aspects that are available to us in mouse models. Such aspects include the ability to look at various brain regions **(Aim 4)** and how each of these affect/reverse brain activity; behavioral

phenotypes **(Aims 3-5)**; determine the critical treatment period when intervention has the largest effect **(Aims 3-5)** and to determine in vivo efficacy and toxicity, including off-target effects **(Aim 5)**. To understand the pathophysiology behind Angelman syndrome we choose to use mouse tissue to answer scientific questions. Depending on the question, for example the role of nuclear UBE3A, we may choose to use a mouse model which only expresses nuclear UBE3A **(Aim 1).**

B. (Optional) Generation of mouse models (Appendix 1): Before we embark on choosing the genes for generating novel GA lines, whenever possible, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation. Although we have generated good mouse models for UBE3A, there may be instances where current available models do not suffice. As an example, 70% of AS patients do not have a mutation that only affects UBE3A, but have a large maternal deletion involving many genes including UBE3A. The question then becomes: how do the neighbouring genes affect the pathophysiology of AS **(Aim 3)**? So far, iPSC derived neurons have not been able to satisfyingly address this question, as the lack of UBE3A in cultured iPSC-derived neurons show little differences. Also, this question cannot be answered using the currently available mouse models and would thus require the generation of new mouse models in which these genes are manipulated in combination with or without a Ube3a deletion. New mouse models can also help to elucidate the role of UBE3A by manipulating genes that encode proteins that are controlled by UBE3A (UBE3A targets) **(Aims 1 & 2)**.

C.Molecular and electrophysiological analyses on tissues/primary cells (Appendix 2): Although we can now generate iPSC-derived neurons, these neurons do not show the same mature properties of neurons as can be obtained from mouse primary brain tissue. Also, electrophysiological measurements require fine-tuned synaptic connections that are not present in neurons grown in a dish, hence we need brain slices to measure the deficits of these neurons. These experiments will help us assess the role of UBE3A at the molecular and cellular level and help us address the questions for **Aims 1-4**. The primary neuronal/astrocytic cultures will be used to test targets or interactors of UBE3A in knock-down or overexpression studies. Also, the efficacy of therapeutics will be tested on brain slices and primary cell cultures prior to any in vivo testing.

D. In vitro selection of therapeutics using mouse primary cells (Appendix 2): After selection of potential therapeutics using mouse cell lines, the most promising lead therapeutics will be tested on primary mouse cultures and brain slices ex-vivo to further test for efficacy in an appropriate cell/tissue type. As an example, electrophysiological recordings in brain slices from mice lacking the entire 15q11-13 locus can help us gain an understanding of the more severe phenotype, more specifically epilepsy, identified in AS patients with a deletion of this region and the contribution of the neighbouring genes on the pathophysiology of AS.

E and F: Mouse behavioural studies (Appendix 3): Once we have identified evidence that a certain therapeutic (which can be a small molecule or a genetics-based therapy) can potentially restore molecular and cellular dysfunction, we would like to test such drugs in mouse models to measure its effects on brain activity and behaviour (**Aim 5**). For that we will typically use our well-established behavioural test battery [6], that is now used by many labs all over the world. We also aim to expand on the behavioural tests that we conduct and introduce new paradigms such as one that measures cognition. In case we have identified a putative UBE3A target (**Aims 1-3**) for which no drug is available, we may also perform a proof of principle study, by crossing AS mice with a mutant of the identified target (reduced- or overexpression) to test the effect of such a double mutant. Behavioural testing is also needed to assess the effect of neighbouring genes in the 15q11-13 gene cluster (**Aim 3**) and to identify which brain regions are underlying the behavioural deficits caused by loss of UBE3A (**Aim 4**).

G. In vivo efficacy using 5.1 lid1c **(Appendix 5):** When a therapeutic works specifically on human cells (eg drugs specifically targeting the human gene such as antisense oligonucleotides) 5.1 lid1c . Besides efficacy, these in vivo studies allow us to test dose and side effects. Such animal studies are needed to safely and effectively

bring drugs to clinical trials (Aim 5).

3.4.2 Provide a justification for the strategy described above.

The strategy is designed in such a way as to:
Keep required animals as low as Examples are to use genetically engineered (immortal) cell lines or iPS cells whenever possible and are, for example, used for our first *in vitro* screening of therapeutics.
Keep discomfort scores as low as The initial work is first performed *in vitro.* This reduces the number of in vivo experiments. For in vivo dose finding we will also use as little animals as possible by first using molecular and cellular readouts (requiring treatment with drug and harvest of tissue). Only when these parameters are established, will we test for behavioural reversibility as well as timing of treatment
Avoid the generation of new animal models when possible. We will not generate and use a mouse model for every AS mutation we are interested 5.1 lid1c

These mice will then be subjected to the therapeutic, and we will solely use molecular and cellular readouts.

3.4.3 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	Generation and breeding of (new) GA mouse lines with possible discomfort
2	Collection of mouse tissue with and without prior treatment
3	Behavioural analysis and advanced phenotyping of treated or untreated mice
4	5.1 lid1c
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Generation and breeding of (new) GA mouse lines with possible discomfort

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.

To understand the fundamental mechanisms underlying the pathophysiology of Angelman syndrome and to perform translational research by devising new treatment strategies, we make use of mice to perform ex-vivo and in vivo experiments. Preferentially, these studies will be carried out on mouse models that already exist. When, after a literature review and also consultations with our extensive international network of partners, a suitable mouse line is not found, we will proceed to design and generate a new genetically modified (GA) line.

De novo generation of new GA lines: If a GA mouse line of a specific gene/mutation is not yet available, we will design and generate the GA mouse line ourselves. We anticipate to **generate a maximum of 5 new lines in the next 5 years**. The genetic modification is applied to embryos or embryonic stem cells, and GA animals are created by:1) injection of GA embryonic stem cells (ES) in blastocysts. 2) Injection of DNA/RNA constructs in oocytes. 3) Modification of oocytes by new techniques for gene editing, such as those employing CRISPR/Cas9.

Breeding of novel GA lines or breeding existing GA lines to obtain novel double-mutant lines of mice with a mild harmful phenotype: Novel GA or existing GA mice with a mild harmful phenotype are bred under SPF conditions to generate mice for research purposes. The novel GA mice will either be crossed with wild-type mouse strains or with other GA mouse lines to generate (novel) double or triple-mutant lines.

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

Table 1 Types of procedures and level of discomfort*)

Vasectomized stud males will be used from the cryopreservation/rederivation program running in the facilityFour procedures involve treatment of the mice: 1) superovulation, 2) implantation 3) vasectomy and 4) distal phalanx clipping or clipping of the ear.

Superovulation: Approximately 5 week old female mice receive 2 i.p hormone treatments, 48h apart. Based on our experience, superovulation at this age results in a maximum number of fertilised oocytes of good quality for most laboratory mouse strains. Superovulation of older mice dramatically reduces the number of oocytes. After the last treatment, the female mouse is mated with a stud male. Next day a plug check is performed. Plugged females will be collected and set aside till usage. All female mice are culled 0.5-3.5 days post mating and embryos are isolated. SOPs apply.

Implantation: Two types of implantations are performed. 1) oocyte/2-cell implantation in the oviduct of a foster mother. 2) blastocyst implantation in the uterus of a foster mother. Surgery is performed on 8-16 week old plugged female mouse (weight 18-30g) following SOP and takes on average 20 minutes per mouse. In brief, a 1 cm incision is made parallel to the dorsal midline to expose the oviduct and uterus.For the oocyte/2 cell implantation: The infundibulum is located and using a fine glass capillary pipet 20-25 oocytes/2-cell embryos are inserted into the oviduct.For the blastocyst implantation: With a syringe a small hole is made in the uterus wall. With a small glass capillary pipet 8-10 blastocysts are inserted through the hole into the uterus.Next the oviduct-uterus is placed back in the abdomen and the peritoneum is closed with 1-2 sutures. Wound clamps are used to close the skin, which are removed 8- 10 days after the operation. Mice are anesthetised and post operation pain relief is administeredAt the 5.1 lid2h , the choice to not super-ovulate embryo donors prior to blastocyst isolation is a conscious one and is based on the fact that in their experience the quality of blastocysts is much lower after superovulation when compared to natural mating. In the end in turns out that a lower number of blastocysts but of higher quality vs higher number of low-quality blastocysts equates to an equal number of mothers used with the added benefit that the discomfort is lower (mild vs moderate). Also, important to note is that with natural matings, only plugged mothers are used.We are also aware of the non-surgical embryo transfer (NSET) procedure which potentially lowers the discomfort of blastocyst recipients. The procedure has been tested in our facility but results in a lower number of pups compared to surgically placing back the blastocysts. So, although the present procedure of surgical implantation of blastocysts does lead to an increased number of donors and foster mothers, ultimately it does reduce the number of repeated injection attempts.

Distal phalanx clipping: Distal phalanx clipping of mouse at 4-5 days after birth following SOPs for identifying and genotypingInitial Welfare Assessment for breeding with a harmful phenotype:As described in the EU directive (2010/63/EU: corrigendum 24-01-2013), various parameters are checked daily. New mouse lines will be monitored for two generations to determine whether there is a harmful phenotype. In those mouse lines that have a harmful phenotype, the breeding of affected animals itself will be registered as (a part of) a procedure. Mouse lines with a more than mild harmful phenotype will not be included in this project.Selection of the most suitable founder lines: Only those newly generated founder lines that have germline transmission AND an appropriate level of GA effect (normally expression of the GA gene in specific tissues/cell types) will be selected to produce offspring for procedures in the other appendixes. Assessment of an appropriate level of GA effect may include (trans)gene (in)activation by eg tamoxifen treatment, continuously or intermittently and via different routes (eg. in max 4 months in feed and/or water; max 5 x IP (5ul/g), or exceptionally - when other methods do not produce the desired result - once ICV (max. 5ul) and subsequent target tissue analyses. Breeding of GA lines with a harmful phenotype (maximum mild severity): some GA lines may demonstrate a harmful phenotype. All breeding strategies will strive for minimizing breeding surplus. However, both for GA lines maintenance as well as production of experimental animals, a GA line with a mild harmful phenotype will need to be bred.

	Gender	Goal	Procedures	Discomfort
1. Donor females	F	Embryo isolation	Mate with fertile stud / Plug check / Cull mouse by cervical dislocation / Harvest embryos, i.e. zygotes, morulae or blastocysts	Mild
		Oocytes isolation	Superovulation (2 i.p. injections, SOP) / Cull mouse by cervical dislocation / Harvest oocytes	Moderate
2. Fertile stud male	M	Fertilization	Mate with female donor mouse (max. 3x per week, 4 months active)	None
3. Vasectomized	M	Mock fertilization	Mate with foster mother (continued use)	Moderate (continued use)

stud male*				
4. Foster mother	F	Oocyte or embryo implantation	Mate with infertile stud male / Plug check next day / Surgical embryo implantation under injection anesthesia and pain relief (SOP) / Birth of GM pups / Wean litter / Cull mouse	Moderate
5. Founders	M & F	Identification DNA extraction and analysis	Distal phalanx clipping of mouse at 4-5 days after birth (SOP)) for identifying and genoptyping. In exceptional cases, an additional clipping of the ears, tail tip clipping or a blood sample is needed for analysis of the genetic alteration.	≤moderate<1%

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

Statistical analyses are typically not being performed for these kinds of experiments, except for testing whether alleles transmit in a Mendelian fashion.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	Mice	N/A (Annex I species specifically bred)	Pups and Adults	1183	Both	Yes	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	Mus musculus: The body of knowledge on the mouse brain is large, and the development of the central nervous system exhibits a high degree of similarity to that of the human CNS, which makes the mouse a good model. Another important factor is the technology to make genetic changes, which allows us to use GA mice as models for Angelman syndrome.
Origin	All animals are bred in the institute or come from a registered supplier and are housed under the same standard conditions. Animals will be socially housed whenever possible
Life stages	Mus musculus: genetically altered (GA) and non-GA animals of defined standard genetic backgrounds, both male and females, typically up to an age of 240 days. Both females and males must be sexually mature to be used in these experiments for them to act as donors (embryo/oocyte/sperm) or as foster mothers. Adult founders will be used for breeding the F1.

Number	Mus musculus: normal (non-GA) animals as well as GA mice of defined standard genetic backgrounds, both male and females, typically up to an age of 240 days. All animals are bred in house or come from a licensed commercial supplier. We estimate to generate a maximum of 5.1 lid1c in the next 5 years. 5.1 lid1c Initial characterisation of the founder lines primarily involves selection on the basis of DNA sequence. This is done on all founders for which often multiple rounds of injection (especially so when point mutations are being introduced via CRISPR/cas9). After selection of the correct founder lines, further characterisation at both the genomic level as well as the protein level is necessary. In our experience with generating GA mice, for example in the case of inducible models, more extensive characterisation of the inducible allele in the offspring from of each founder is necessary and how this affects protein levels needs to explored. This involves the treatment of animals with eg. Tamoxifen or crosses with Cre-expressing lines to obtain tissues to test on Western blot, immunofluorescence labelling etc. We would like to stress that treatment with therapeutics is not done at this stage. Also important to indicate here, is that for some mouse models, for example ones in which we introduce simple point mutations, although getting these into the genome via CRISPR/Cas9 is quite a difficult endeavour, sequencing of DNA obtained from toe clips from the founder and F1 mice will suffice in determining which of the founders/F1 are correctly targeted. For these types of mutant mice, extra mice for extensive characterisation may not be necessary. Therefore the 50 mice requested here for extensive characterisation per generated line is a maximum number of mice needed and the numbers for each line will always be justified in the study-plan submitted to the IvD prior to performing any experiments. 5.1 lid1c are humanely killed to collect tissue for a more extensive analysis to select the correct founder line, using for example Western blot, immunofluorescence or QPCR (to check for the correct change in protein levels/ DNA). Since we will be generating mice harbouring a loss of function or dominant negative mutant allele of a NDD gene, some of the newly generated heterozygous GA mice may be born with a mild harmful phenotype. To test these mice in the different assays as mentioned in appendix 2 and 3, we may need to breed mice with a mild harmful phenotype. 5.1 lid1c
Gender	For the majority of experiments, mice of both genders can be used. There are exceptions such as egg donors and fosters being female and vasectomised males being male for obvious reasons.
Genetic alterations	For the generation of mouse models, it is important that the mutation encountered in patients is also introduced into the mouse genome to create a model with a high construct validity. This in turn increases its face validity. It is not possible to mention which mutations will be modelled here but will be identified in the study-plans.

Strain	We will use normal (non-GA), genetically altered (GA) and wild-type control mice in different standard genetic backgrounds (eg Bl6, 129 and FVB) or combinations (eg F1 Bl6 and 129) thereof depending on how the genetic background affects the phenotypes we see in the strains. The strains to be used will be mentioned in future study-plans.

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

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

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

Surgical procedures, including embryo transfer and vasectomy, will be carried out under adequate perioperative analgesia and general anaesthesia

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

In many cases it is difficult to predict exactly what the effect will be of an alteration of the DNA sequence of the mouse genome. From our experience, none of our AS mouse models (5.1 lid2h) show any deleterious effect on their well-being, and phenotypic changes we measure are only observed through advanced experimental testing where specific phenotypic modifications can be detected. However, since we may also generate models involving genes other than Ube3a, in 40% of the newly generated GA lines we can potentially expect a mild harmful phenotype such as susceptibility to epilepsy. It is difficult to predict beforehand the severity of the phenotype we expect as this can differ between humans and mice. When mouse lines do experience an intrinsic discomfort severity beyond mild, the line will no longer be used and the generation of an inducible line will be considered.

Explain why these effects may emerge.

These effects may emerge because of the role of the gene in brain development

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

If unexpected adverse effects are observed, immediate action will be taken. This could be through removal of a specific animal from the experiment, or even termination of the entire GA mouse line in favour of a more refined model (e.g. inducible mutant).

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

All mice will be observed daily with respect to several parameters (overall appearance, size, confirmation and growth, coat condition, behaviour, clinical signs, relative size and numbers) as has been described in

the Directive 2010/63/EU: corrigendum of 24 Jan. 2013. All animals that score more than mild discomfort will be euthanised, with the exception of animals recovering from surgery (maximum discomfort: moderate).Animals that underwent surgery (fosters/vasectomised males) will be euthanised if they meet one or more of the following criteria:-The animals stop eating or drinking.-If there is a decrease of body weight after the surgery (10% relative to their weight at the start of the experiment)-If there are moderate circulatory or respiratory problems, or if they develop a neurological disease that results in suffering (eg sustained deviant behaviour such as abnormal aggression, epilepsy, lethargy).In some instances, the mouse line may have an increased susceptibility to epileptic seizures. This could be only when this is induced will a loud sound (eg Angelman syndrome mice). In other instances, it may involve an increase in spontaneous seizures. Depending on the gene or mutation involved this may only occur if the mice are homozygous. In such situations only for experiments will homozygous animals be bred. It is important to mention that all mice that display an intrinsic discomfort level >mild will not be used. All such details will be described in the study-plans.

Indicate the likely incidence.

From our extensive experience with generating new GA lines in the last 20 years, it is not common to observe these events. We estimate this to be less than 10%.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Severity	#	%	Treatment Groups
3 Moderate	5.1 lid1c		Female donors (oocyte and embryo isolation)
2 Mild			Vasectomized stud males (will be used from cryopreservation/rederivation program)
3 Moderate			Foster moms
2 Mild			Founders
2 Mild			issue collection for analysis F1
2 Mild			breeding mice with a mild phenotype

Donors: multiple injection of hormones to induce super-ovulation. Humanely sacrificed and eggs harvested (severity: Mild)

Fosters: These mice will undergo abdominal surgery to place back the blastocysts or injected eggs. Humanely sacrificed after weaning (Moderate) Vasectomised males: Males undergo abdominal surgery for sterilisation purposes (Moderate)

Offspring and breeding: These mice do not undergo any treatment. There is a potential for breeding with a mild harmful phenotype but that is difficult to predict (Severity: mild). The emergence of a mild harmful phenotype depends on the protein involved, and can cause for example, mothers to be more prone to stress and becoming more aggressive. As a consequence, some of the pups may be cannibalised by the mother within the first 14 days after birth. Mutations introduced into some mouse lines may result in an increased seizure susceptibility the frequency of which is at most 5/day This is determined during the extensive analysis of each line when mice will be observed with cameras and footage analysed to detect the presence of seizures. When the severity of the experienced harmful phenotype is deemed too high, the mouse line will no longer be used and the generation of an inducible mouse model may be considered (Severity: Mild).

All mice are humanely sacrificed

G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to modify the gene and to study the effect of the genetic modification on brain development and brain function. To date, studying GA mice is the only reliable method to investigate the function of a gene in brain development and brain function *in vivo*. Before we embark on choosing the genes for generating novel GA lines, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation.
Reduction	The vasectomised males used in this study also participate in the cryopreservation/rederivation program that is ongoing in the transgenic core facility, thereby obviating the need to use a separate cohort of mice.
Refinement	The lab has obtained extensive experience in the last 2 decades in the type of procedures been described and knows how to determine when mice are approaching humane end points such as weight loss, trembling, fur condition etc. The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of neurodevelopmental disorders, and participate in scientific meetings. Also, we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study. We continuously strive to reduce the discomfort the mice are subjected to, and although some labs do use the NSET procedure, a non-surgical procedure to place blastocysts back into the recipient, experience with this procedure in our facility resulted in a lower number of pups compared to when we used the surgical procedure, described in this application. We do recognise that the present procedure of surgical implantation of blastocysts does involve a higher discomfort to the mice, we believe that the higher number of mice/injection obtained in our hands does ultimately reduce the number of repeated injection attempts.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

A number of the animals will be humanely killed after general anesthesia, eg. egg donors and foster mothers (at weaning) in the course of the experiment. Non-GA offspring will be killed before weaning.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☒ No > Describe the method of killing.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia. This causes least pain andstress for the animals

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons or to resolve welfare concerns.

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 3 — Type of animal procedure: Collection of mouse tissue with and without prior treatment

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.

To address the research questions of this application, we will make use of *ex-vivo* experiments as much as possible. This appendix describes the collection of

1) embryonic brain tissue to setup primary neuronal cultures to be used to determine the role of UBE3A protein in the different cellular compartments (**aims 1 and 2**) and to determine the effect of AS mutations or neighbouring genes, or the deletion thereof, have on molecular readouts of AS (**aim 3**).

2) These same questions will also be investigated at different ages in vivo by collecting brain tissue and subjecting these to various biochemical and electrophysiological analyses (**aims 1-3**). In addition, the identification and effects of therapeutics in the various AS lines, both current and to be generated, will be looked at (**aims 4 and 5**) to assess the *in vivo* effects of therapeutics at the molecular and cellular level, for molecular analysis or slice electrophysiology. To address these aims, we will use normal and GA (single, double or triple) mice. In order to obtain the tissues, animals are humanely killed after general anaesthesia, and tissues are dissected for further analyses using biochemical/molecular, electrophysiological and imaging methods (microscopy analyses). For **aims 3 and 5,** we may 5.1 lid1c a transcriptional enhancer or inhibitor (to modulate specific gene expression levels), small molecules or vehicle to the animals before we collect the tissue.

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

Treatment of animals: The animals may be subjected to drugs that activate/inactivate the gene/allele **(induction)**. For example, the animals may receive maximally 5 IP injections of tamoxifen (maximum volume of 5ul/g) to induce brain-specific gene deletion/activation in the case of floxed alleles. They may receive the therapeutic drug to be tested at E14.5 to >P21.

Therapeutics may be administered to embryos via **ICV** (intracerebroventriuclar) or neonates via **IP, IV, SC, ICV, intracerebral or orally** and to older mice via **IP, IV, SC, ICV, oral, cannula or via osmotic pumps**. Treatment will be alone or in combination (one treatment regimen per mouse), continuously or intermittently by one or more routes (for example in food and/or water; subcutaneously, intraperitoneally, oral gavage (max. 2x daily, for 8 weeks), implanted with an osmotic pump (1x) or via intrathecal or ICV injection or via a cannula. These latter three procedures, including the implantation of the cannula are done only once/mouse and require surgery with adequate general anesthesia and perioperative analgesia. As most drugs do not persist beyond 16 weeks, treatment will be for a max. of 20 weeks (max. 4 weeks preceding the testing and 16 weeks during testing). A subset of animals may receive perinatal drug treatment, which we will administer (IP, oral gavage or subcutaneous) to the pregnant or lactating dams. Another subset of animals may receive treatment in utero, for which the drug will be administrated to the embryos by intraventricular injection, for which we need to perform surgery on the pregnant mothers. A pregnant dam will be operated aseptically under peri-operative analgesia and general anaesthesia at 16-19 days of timed pregnancy (third trimester), and the uterus is carefully exposed. Each of the foetuses will be injected with the drug in the lateral ventricles leaving the uterus intact. After this, the uterus is carefully placed back into the abdominal cavity and the abdominal incision will be closed by multiple layers of sutures. The total surgery time is maximally 30 min, from the moment the dam is anesthetised until she wakes up again, and 5-6 days later pups will be born naturally. The dam will be sacrificed after weaning without undergoing any other procedures. The animals are randomly selected for the respective drug treatments to be tested and the handler/observer (tester) is blinded for the specific genotype or treatment. All animals are humanely killed after testing to obtain tissue for analysis.
All individual treatments impart **maximally** a moderate discomfort to the animals, with the exception of the addition of therapeutic to drinking water or food. Currently our most effective route of administration of therapeutic is via a single bolus ICV injection at all life stages (In utero, neonates and >P21) since molecules such as ASOs, small molecules and most viruses, do not pass through the blood-brain barrier. We estimate that 75% of our mice will be treated via this route. Due to the invasive nature of IVC injections, we aim to investigate the possibility of systemic administration, via IP, SC, IV for instance. It is important to note that the cumulative discomfort endured by each animal due to the treatment(s) should never exceed moderate.

Tissue collection of foetuses (No treatment): Since neurons are post-mitotic (do not divide) we need primary cells for our neuronal cultures. To derive these cells, time-mated pregnant mothers are killed after general anaesthesia to obtain foetuses from which hippocampal and/or cortical cultures are set up. For glial cultures, P0-4 pups are sacrificed and the brain tissue used to set up cell cultures. These cells allow us to over-express or knock-down a myriad of proteins and determine whether they cause changes associated with the mutated protein in question in terms of morphology, protein expression and phosphorylation levels etc. The mothers are sacrificed as part of the experiment and do not undergo any additional procedure. The time-mated pregnant mothers are humanely killed after general anaesthesia. The uterus with foetuses is rapidly cooled on ice water to provide anaesthesia, and the foetuses are successively decapitated to obtain the brain for the hippocampal and/or cortical cultures. For glial cultures, the P0-4 pups are subjected to hypothermia-anaesthesia (pup placed in "finger glove" and rapidly cooled in ice cold water), decapitated and brain tissue isolated.

Tissue collection of post-natal/adult animals with or without treatment: Mice may receive a single or multiple IP injections of therapeutics (maximum volume of 5ul/g), via single or multiple IV injections (maximum volume 10ul/g), via single or multiple SC injections (maximum 10ul/g) or a single or multiple doses via oral gavage (maximum volume 10ul/g). The number of treatments depend on such factors as the half-life or rate of clearance of the therapeutic from the body. Each of the aforementioned treatment routes results in a mild discomfort/treatment but will result in a cumulative maximum discomfort of moderate when multiple injections are given. Treatment may also consist of a single ICV injection (maximum 10ul) or a single or multiple doses via oral gavage (maximum volume 10ul/g). In the event multiple treatments are necessary via the CSF, a cannula is placed during a craniotomy under general anaesthesia and the therapeutic administered during the cannula placement and after the animals have recuperated (maximum volume 3.5 ul). When a slow infusion of the therapeutic is necessary (Maximum volume of 0.25 or 0.15ul/hr for resp. 4 or 6 weeks) an osmotic pump in placed subcutaneously on the back slightly posterior to the scapulae and connected to a brain cannula placed with via a craniotomy. All this is done under general anaesthesia. The exact mode of administration, frequency and volumes of therapeutics will be indicated and

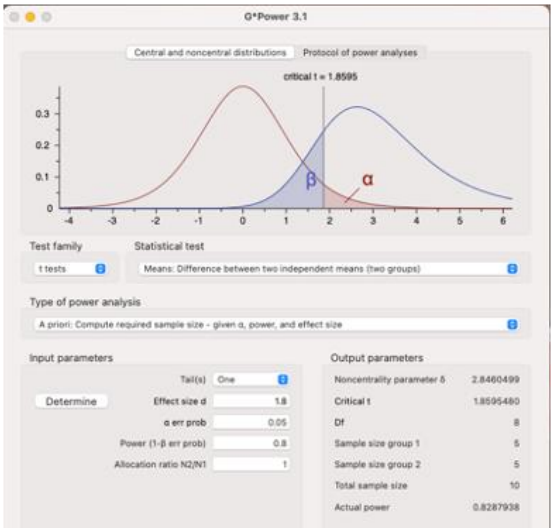
justified in the study-plans that will be assessed by the IvD .To obtain (brain) tissue, mice will be humanely killed at specific time points. This may be after gene deletion/activation (if applicable) in addition to treatment with the therapeutic in question or with vehicle and their brain tissue will be isolated to be used for a variety of experiments. Killing of animals is always performed after providing appropriate general anaesthesia.

Tissue collection following prenatal treatment (requiring surgery of dams): A subset of animals may receive prenatal (*in utero*) treatment of therapeutics and the effect thereof determined via molecular, electrophysiological or imaging means. Another group will be treated with plasmids expressing specific genes of interest or specific patient mutations where we look at how the over expression or knockdown of expression affects the migration of brain cells throughout the brain after birth.The therapeutics or plasmids will be administrated to the pups via a single intraventricular injection (maximum volume of 2ul), for which we need to perform surgery on the pregnant mothers. A pregnant dam will be operated aseptically under peri-operative analgesia and general anaesthesia at 14-17 days of timed pregnancy, and the uterus carefully exposed. Each of the foetuses will be injected with the therapeutic in the lateral ventricles leaving the uterus intact. In case of plasmid delivery to cells, we will apply 5 precisely dosed short electrical pulses using an electroporator (in utero electroporation (IUE)), to move the (charged) DNA into the neuronal precursor cells that are located in the subventricular zone. After this, the uterus is carefully placed back into the abdominal cavity and the abdominal incision will be closed by multiple layers of sutures. The total surgery time is maximally 30 min, from the moment the dam is anaesthetised until she wakes up again, and 5-6 days later pups will be born naturally. From extensive experience with this procedure, only very rarely (±5%) do the dams encounter issues during birthing. After weaning the dam will be humanely killed after general anaesthesia.

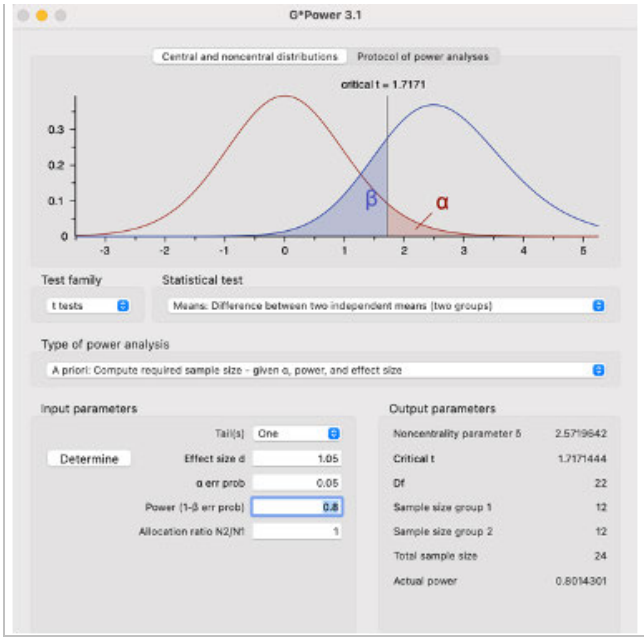
Tissue collection of new-born animals: In case we need to collect tissue from new-born mice for subsequent analysis, pups (P0-P5) are rapidly cooled on ice water to provide hypothermia-anaesthesia, and decapitated to obtain the brain for the further analyses.

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

We use tissue for a large variety of experiments, each requiring different analyses and sample sizes. In most cases the number of animals will be based on literature and/or previous experience with similar experiments. Such experiments will be carried out using a minimum number of mice per group to detect meaningful effect sizes (β of .8 and an alpha (α) of 0.05). The numbers are mostly based on previous experience and strongly depend on the outcome measure. The experimental design including numbers of animals will be submitted to the IvD. Only mice with the desired genotype will be used with the exception of embryos and P0-P3 pups when the whole litter is treated because genotype is unknown at time of treatment.As an example, for molecular analyses, we use 5 samples/group (90-95% survival) = 6 mice/group.For electrophysiological analyses, 12 animals/group is used based on historical data and the fact that a limited number of cells can be patched/brain slice.



For electrophysiological analyses, 12 animals/group is used based on historical data and the fact that a limited number of cells can be patched/brain slice.



B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	Mice	N/A (Annex I species specifically bred)	Pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	*Mus musculus*: Knowledge about the mouse brain is large, and the development of the central nervous system exhibits a high degree of similarity to that of the human CNS, which makes the mouse a good model. Another important factor is the technology to make genetic changes, which allows us to use GA mice as models for neurodevelopmental disorders.
Origin	All animals are bred in the institute or come from a registered supplier and are socially housed, whenever possible, under the same standard conditions. This ensures the animals are genetically pure and free of known infectious agents.
Life stages	Mice at all stages (from E14.5, neonates, juvenile, adolescent or adult) will be subject to biochemical, immunohistochemical, ePhys analyses after treatment with therapeutic/vehicle. This allows us to properly study the different developmental stages of the mice. The chosen age will depend on the functional test and whether the age is suitable for the test. The treatments described for the induction of the GA alleles and/or treatments with drugs will in some cases begin before parturition, to study the effects on the embryos during development.
Number	The numbers detailed below are a maximum we will expect to use. The actual numbers and conditions will be outlined and justified in the study-plans presented to the IvD for consent prior to performing any experiment. In the proposed experiments we will make use of a maximum of 6 GA mouse lines. Below we delineate the number of mice needed for each type of experiment. **Neuronal cultures (aims 1 and 2):** In Aims 1 and 2 we look into how specific mutations lead to a change in localisation and

how UBE3A interacts with its "targets" in addition to deciphering the role of UBE3A in the nucleus/cytosol/synaptic compartments. To answer the questions using neuronal cultures, we expect to test a maximum 5.1 lid1c . On average, normal (non-GA) or GA pregnant mice provide enough foetal cells to carry our 2 measurements. 5.1 lid1c

To assess potential therapeutics in neuronal cultures we expect to test a maximum of 6 GA lines + controls. For each mouse line, we need 5.1 lid1c different sequences and chemical modifications are used in the different candidate compounds and the efficacy and possible off-target toxicity is determined at this stage. To determine the sample size we need to show a difference of a factor 2 in eg. protein or mRNA levels between WT and mutant lines, using G*Power with an a=0.05 and a power of 80%, we calculate a sample size of 5. 5.1 lid1c

Treatment of embryos requiring surgery of dams: We estimate that maximally 4 interventions will be initiated before parturition and will be administered by intraventricular injection of embryos, for which we need to perform surgery on the dams. 5.1 lid1c

In utero electroporation (IUE): a maximum of 25 patient derived mutations will be studied in addition to a maximum of 25 candidate interactors/regulators in the next 5 years. Per *Ube3a* 5.1 lid1c *Ube3a* 5.1 lid1c . A control plasmid is not needed every single time, and will be included per 20 mutations/interactors/regulators/knockdown, 5.1 lid1c *Ube3a* 5.1 lid1c

In the course of 5 years, additional staff (BSc and MSc students, technicians, PhD students, postdocs, investigators of other institutions) will need to be trained for this technique (one person per year) From experience we know that it takes 10 dams to fully master the technique, thus we will need in total 10 x 5 years = **50 dams**.
In case all goes well, the dams will be allowed to carry to term and the animals will be used for analysis. Considering that a dam on average caries 6 embryo's, this means a maximum of **300 pups** involved to investigate if the electroporation was performed correctly. We will strive to use the training mice for obtaining useful data to reduce the number animals used.

Molecular analyses (Aims 1-4):

	To perform a detailed biochemical (e.g. proteomics, Western blots, kinase assays), genetic (e.g. gene expression (RNA-seq) analysis) and imaging analysis (e.g. immunofluorescence) on therapeutic or genetically manipulated WT or GA mice, we estimate to maximally require:5 interventions 5.1 lid1c The group size of 6 mice here is based on the 5/group explained above and taking into account 95-90% survival upon treatment. **Electrophysiological analyses with or without treatment (Aims 2-5):** To address these aims (Role of UBE3A localisation, interaction with targets, effect of neighbouring genes and discover specific brain regions involved in AS-specific phenotypes) in addition to testing the effect of certain interventions and further characterisation of new models, we will sacrifice the animals and perform slice electrophysiology and record a number of different electrophysiological parameters from different cell-types, different brain areas, and different developmental ages. There are many parameters we can measure and we can measure them from distinct cell-types and brain areas. At this point it is hard to make an accurate plan of the parameters we will measure in the next 5 years. We expect to do a detailed electrophysiological assessment of at most 6 lines for which we will typically need 12 animals/group (WT/mutant; treated/untreated; or in case of Floxed mice we will have 4 genotypes) and in which we will assess 3 parameters after treatment with vehicle or a therapeutic or 3 parameters after Cre activation. The choice of 12 animals/group is based on historical data and the fact that a limited number of cells can be patched/brain slice. Again, we stress that individual study plans will be sent to the IvD for their endorsement prior to performing any animal experiments. 5.1 lid1c Whenever possible, we will perform molecular experiments on the tissue that have been used for electrophysiological experiments. This may be possible for morphological experiments. However, in many cases, the electrophysiological experiments will reduce the quality of the tissue to such an extent that they cannot be used for other molecular experiments such as gene expression studies.
Gender	Most phenotypes do not differ between male and female patients and mice, and hence we will use both sexes. In the event there is a gender bias, we will choose the gender with the strongest phenotype.
Genetic alterations	For our research, it is vital that we use mouse models that carry mutations similar to those encountered in patients giving the models a high construct validity which in turn translates to a high face validity.
Strain	We will use normal (non-GA) control and genetically altered (GA) mice in different standard genetic backgrounds (eg Bl6, 129 and FVB) or combinations (eg F1 Bl6 and 129) thereof depending on how the genetic background affects the phenotypes we see in the strains.

C. Accommodation and care
Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?
☒ Yes
☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

The discomfort depends on how the therapeutics are administered. This can be through IP, SC or IV injections without the use of anaesthesia. When mice are injected ICV or intracerebrally, hypothermia-anaesthesia (P0-P3) is applied. For implantation of an osmotic pump (1x) or administration via intrathecal or intracerebroventriuclar (ICV) injection or via a cannula, the procedure is carried out only once/mouse and requires surgery with adequate general anaesthesia and perioperative analgesia.

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

Treatment with therapeutics can potentially have unforeseen side effects. Also, it is possible that the animals do not recover well from surgery after ICV or intracerebral injections, implantation of a mini pump, or from oral gavage or injection (intraperitoneal, intraventricular, intravenous or subcutaneous) of the therapeutic. Also, complications such as infections at the site of injection, cannula/pump may occur. Most GA animals will not have a harmful phenotype, but we estimate that maximally 2 GA lines may show a mild harmful phenotype.

Although, from our experience, most genetic modifications have no effect on the well-being of the animal and changes are only seen through advanced experimental testing. In some cases where harmful phenotypes are expected, the generation of inducible models may be the solution. In many cases it is difficult to predict exactly what the effect will be of an alteration of the DNA sequence of the mouse genome. From our experience, most genetic modifications themselves have no effect on the well-being of the animal, and phenotypic changes are only observed through advanced experimental testing where specific phenotypic modifications can be detected. However, in 40% of the newly generated GA lines we can potentially expect a mild harmful phenotype such as susceptibility to epilepsy. It is difficult to predict beforehand the severity of the phenotype we expect as this can differ between humans and mice. In some cases where harmful phenotypes are expected, the generation of inducible models may be the solution.

5.1 lid1c

Explain why these effects may emerge.

Some of the new lines have not been characterised and although the impact of most procedures on animal welfare are well known, the effect of the therapeutics implemented are unknown and may elicit side effects.

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

If unexpected adverse effects are observed, immediate action will be taken. E.g., the drug dose will be reduced or treatment may be withdrawn altogether. If appropriate we will administer pain medication or remove the animal from the experiment and euthanise it.

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:

1. The animal stops eating or drinking.
2. Decrease of body weight of 10% relative to their weight at the start of the experiment, stunted growth before onset of the experiment (15% reduced compared to littermates).
3. Moderate circulatory or respiratory problems.
4. The development of a clinical neurological disease that results in sustained suffering (eg they display deviant behaviour such as abnormal aggression, epilepsy, lethargy). When the animal experiences 20 or more seizures/day), lethargy).

In some instances, the mouse line may have increased susceptibility to epileptic seizures. This could be only when this is induced by a loud sound (eg Angelman syndrome mice). Depending on the gene or mutation involved this may only occur if the mice are homozygous. In such instances breedings will be such that only for experiments will homozygous animals be bred.

In the event that mice experience any discomfort due to the treatment per se, or during the entire experiment such that the cumulative discomfort rises beyond "moderate", the affected animals will be taken out of experiment.If a mouse line displays an intrinsic discomfort beyond mild, the line in question will be discontinued and it may be decided to generate an inducible model instead.

All such details will be described in the study-plans.

Indicate the likely incidence.

With our extensive experience with generating new GA lines in the last 20 years, it is not common to observe the above mentioned events. We estimate this to be less than 10%.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Severity	#	%	Treatment Groups
2 Mild	5.1 lid1c		euronal cultures (dams)
3 Moderate			lectrophysiology
3 Moderate			olecular analysis
3 Moderate			reatment of embryos
3 Moderate			reatment of embryos requiring surgery of dams
2 Mild			euronal cultures (embryos/P0-1 pups)

Neuronal cultures (dams and embryos, pups): Both mother and embryos are sacrificed humanely without any treatment (Severity: Mild).

Depending on the route of administration, the mice used for molecular and electrophysiological analyses will receive the treatment (eg IP, IV, SC) without surgery (severity: Mild/single treatment) to cumulative moderate maximum with repeated injections), or they will receive the treatment during a surgical procedure (eg. ICV injections [embryos, pups and adults], pump, via cannula or other invasive route) (severity: moderate).

For some new lines, it may involve breeding with a mild harmful phenotype but that is difficult to predict (Severity: mild). At most 10% of the mice in this appendix involves breeding with a mild harmful phenotype, which, due to the mutation, may result in an, for example, increased seizure susceptibility.

G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to study the effect of the genetic modification on brain development and brain function. To date, studying GA mice is the only reliable method to investigate the function of a gene in brain development and brain function *in vivo*.
Reduction	Before we embark on choosing the genes for generating novel GA lines, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation. We also employ some mice for *in utero* manipulation

	of the brain to determine whether this causes a phenotype. Once we decide to test a therapeutic, this is first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when the therapeutic is found to function well in in vitro and ex-vivo experiments, will it be tested in vivo, which reduces the number of animals we use.
Refinement	The lab has obtained extensive experience in the last 2 decades in the type of procedures been described and knows how to determine when mice are approaching humane end points such as weight loss, trembling, fur condition etc. The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of neurodevelopmental disorders, and participate in scientific meetings. Also we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons or to resolve welfare concerns.

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 3 — Type of animal procedure: Behavioural analysis and advanced phenotyping of treated or untreated mice.

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

Mouse models for AS often recapitulate the main features of NDDs: **intellectual disability, abnormal behaviour, motor deficits, sleep deficits, abnormal EEG and epilepsy**. This research concerns the behavioural analyses performed on existing or newly generated (**Appendix 1**) AS mouse models using behavioural tests in the presence or absence of treatment. This appendix describes a standardized behavioural test battery that was optimized and implemented in the lab to efficiently and objectively assess phenotypes as observed in AS mice but also includes additional tests that may yield a phenotypic difference between WT and mutant littermates. New and existing mouse models will be subjected to these tests to establish whether or not phenotypic differences are observed and can be used as a functional assay to determine the effect of genetic, therapeutic or pharmacological intervention. These experiments will help address **aim 1** (role of nuclear UBE3A), **aim 2** (function of UBE3A in cytosol and synapse), **aim 3** (role of neighbouring genes on AS pathophysiology), **aim 4** (identifying brain region underlying behavioural deficits) and **aim 5:** Identify therapies that ameliorate the behavioural deficits of AS mice.

GA mutant mice and their WT littermate controls: GA mice include heterozygous mutation bearing or mutant heterozygous knockout mice for specific genes suspected to play a role in AS, or a combination of floxed mutant GA mice with Cre-expressing mouse lines; or a mutant crossed with a reporter gene; GA and their WT littermate control mice treated with agonists or antagonists of pathways for which there is evidence that they are affected in AS; GA and their WT littermate control mice injected (via sc, IP, IV, ICV, intracerebral injections, cannula, oral gavage or implanted osmotic pump) or in other relevant brain areas with therapeutics.

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

Below are examples of behavioural assays that mutant and WT littermates may be subjected to.

Test battery for gross motor performance and innate behaviours:

We have setup a behavioural test battery which probes motor issues, and a number of innate behaviours such as anxiety issues and behaviours that are often affected in AS mouse models. For the tests described below, a single cohort of (treated) mice, comprising of mutant and WT control mice, will be subjected to a maximum of 7 tasks for which we have demonstrated a difference in performance between WT and mutant mice. After extensive handling of the mice to decrease stress and to get the animals acquainted with the experimenters, all mice in the cohort are sequentially subjected to these tasks over a period of 2-4 weeks. The most stressful task (5.1 lid1c (highest discomfort score) is done last, so as to not interfere with the outcome of the earlier tests. After the behavioural testing, the mice are humanely killed to analyse the brain tissue. The animals are carefully acquainted to handling before they testing ensues to minimize stress levels. The animals are randomly selected to be tested and the handler/observer (tester) is blinded for the specific genotype or treatment.

Hind limb clasping reflex (max 10s, twice)(Severity: mild). Hind limb clasping is a marker of motor impairment and cerebellar dysfunction. The clasping test evaluates the animal's hind limb response during tail suspension 10 cm above the home cage. If the hind limbs are splayed out- ward, away from the abdomen, the mouse is assigned a score of 0. If one hind limb is retracted toward the abdomen, the animal receives a score of 1. If both hind limbs are partially retracted towards the abdomen, it receives a score of 2. The animal receives a score of 3 if the animal's hind limbs are entirely retracted and touching the abdomen.

Wire hang test (max. 60s once) (Severity: mild). The Wire hang test is used to measure subacute muscle function and fatigue. A horizontal wire (2 mm in diameter, 40 cm in length) is suspended 20 cm above a padded table. The animal is positioned to cling in the middle of the wire with its forepaws for one 60 s trial, and latency to fall is recorded.

Accelerating Rotarod or Accelerating Reverse Rotarod (2 trials of max. 5 min a day, for max. 5 days) (Severity: mild). This test measures motor coordination/performance. Mice are tested 2 times a day (ITI 1hour) on the Rotarod (a turning accelerating cylinder with a diameter of 3 cm (accelerated 4-40 rpm). This is repeated over a total of 5 days. The outcome parameter is the amount of time the mice can stay on the rod without falling. Maximum time per trial is 5 min. The reverse rotarod is the same as the accelerating Rotarod with the exception that the mice are placed on the rotating drum in the opposite direction, forcing them to walk backwards. This is a more demanding task than the regular accelerating Rotarod, and depending on the severity of the motor impairments we choose either one of these rotarod paradigms.

Open field (max. 3x 10 min) (Severity: mild). This test measures anxiety and general activity. Mice are placed individually in a brightly lit 120 cm diameter circular open field for a period of 10 minutes and their exploratory behaviour is recorded on video and analysed by tracking software.

Marble burying (max. 30 min once) (Severity: mild). This test measures anxiety/repetitive behaviour. Mice are placed for 30 min. in a clean polycarbonate cage with a rich amount of bedding material. On top of the bedding material 20 blue glass marbles are arranged in an equidistant 5 x 4 grid and the animals are given access to the marbles for 30 minutes. Most (WT) mice will try to bury the marbles. The outcome is the number of marbles buried (visually inspected).

Nest building (max. 7 days continuous) (Severity: mild). This test examines the nest building behaviour of a singly housed mouse over a period of maximally 7 days. On day 1 of the test 3 cotton squares (nestlets) are added to the cage to be used by the mouse to build a nest. The weight of the remaining unused nestlets is determined each morning for maximally 7 days, allowing for the quantification of the nest building behaviour.

5.1 lid1c

5.1 lid1c

Cognitive tests:
These tests are very important to study whether treatment of GA mice with therapeutics rescues cognitive abilities relative to the WT litter mates. All tests described below require a **separate cohort** of mice.

Morris Water maze (max. 2 times daily for 1 min; for 14 days) The water maze test is a very sensitive test to probe spatial learning deficits. In order to reduce the stress levels, a week prior to the start mice will be handled every day for 2-5 minutes. Handling consists of picking up the mouse and putting it on the hand or sleeve. Adaptation will show by a more relaxed behaviour and some exploratory initiatives. This reduces the stress levels of the animals during the water maze test and consequently reduces the number of mice that have to be excluded from this test because of under-performance. During training the mice will receive 2 trials a day for 60 sec to find the hidden platform just beneath the surface of a pool (diameter 1.20 meters) filled with visually opaque warm water (temp 25-26 degrees Celsius). Visual cues, such as coloured shapes or patterns, are placed around the pool in plain sight of the animal. After 1 min of swimming has passed the mouse will be placed on the platform for 30 sec. This will be repeated 5-14 days depending on the learning curve (finding the platform). At the end of the training the mice will get a probe trial in which the platform is removed to see if the mouse has learned the location of the platform. Various parameters are measured including average distance to platform, latency to escape, path length, and velocity.

Barnes maze (max. 2 times 15 min; for max 14 days). The Barnes maze consists of a circular surface with 20-40 circular holes around its circumference. Visual cues, such as coloured shapes or patterns, are placed around the table in plain sight of the animal. The table surface is brightly lit. Underneath one of the holes is an "escape box" which can be reached by the animal through the corresponding hole in the table top. The model is based on rodents' aversion of open spaces, which motivates the test subject to seek shelter in the escape box. A mouse will typically learn to find the escape box within four to five trials and in subsequent trials will head directly toward the escape box without attempting to escape via incorrect holes. Various parameters are measured including latency to escape, path length, number of errors, and velocity.

T-maze (max. 10 times 1 min; for 7 days): A T-maze is a simple maze used in animal cognition experiments. It is shaped like the letter T (or Y), providing the mouse with a straightforward choice. T-mazes are used to study rodent learning and flexibility when the task is modified. The animals are briefly (overnight) food deprived prior to the test and learn the test through positive reinforcement (food rewards). They can enter and leave both arms and the learning outcome is the immediate proper choice between the arms.

Tests designed to study social deficits:

These tests are very important to study the role of a gene/protein in autism. If the mutation in question is linked in humans to autism, GA mice carrying the mutation may be subjected to these tests in an effort to determine the effect of treatment in reversing autism phenotype. All tests described below require a **separate cohort** of mice.

Three chamber test: (max. 1 hour; daily for 5 days). This test measures how much a test mouse interacts with a stimulus mouse (known or unknown) as compared to interaction with an object. The test mouse is placed in the centre compartment (43x40 cm) of the three chamber set up, and is monitored to explore the outer compartments containing either an object or a confined stimulus mouse.

Social hierarchy (tube) test: (max. 1 hour; daily for 7 days). The mice are trained (using an air-puff as a mild adverse stimulus) to leave the entry box and walk through a narrow tube to the opposite box. After training, one mouse is placed in the entry box and another (unknown) mouse is placed in the opposite box. When the doors are opened, the mice will encounter each other in the middle, and one of the mice will have to move backwards. These movements are recorded and analysed. Depending on the precise research question, the mice will be housed in mutant/wt pairs (for mutant x wt matches), or single housed (for hierarchy matches in which each mouse encounters all other mice).

Tests measuring brain activity and epilepsy: Also for both 5.1 lid1c **and audiogenic seizure tests, a separate cohort of mice are needed**

5.1 lid1c

Sensitivity to Audiogenic seizures (Max 20s twice): To determine the susceptibility of mice to audiogenic seizures, both mutant and WT littermates are subjected to the noise made by vigorously scraping scissors across the metal grating of a cage lid, which generates a 100dB noise. This is done for 20 seconds, or less if a tonic-clonic seizure develops before that time. A typical seizure lasts 5-15s, and the animals appear fully recovered within 30 seconds. This test is done at the start of an experiment (baseline) and if needed repeated one more time after a specific treatment.

Introduction of novel testing paradigms
It is possible that during these 5 years we may introduce a test that is not described here for testing AS mice. In particular in the domain of social tests (autism) and cognitive tests (learning disability) there is a need for better (more robust, less intense) tests. Such test will not exceed the discomfort of the tests described here, and will only be considered if it provides a clear scientific advance or is better with respect to reduction or refinement.

Administration of drugs: For a number of experiments where inducible mice are to be used, activation of the Cre protein will be achieved through treatment with tamoxifen. This mostly occurs via IP injections but there may be the need to inject directly into the ventricles to initiate efficient Cre-mediated recombination in utero or shortly after birth (P1-3). For the intervention studies, at most 4 compounds will be tested in mice at all stages of development to determine if there is a reversal in 1 or more behavioural phenotypes. Therapeutics to be administered include 5.1 lid1c They may be administered alone or in combination (one treatment regimen per mouse), continuously or intermittently by one or more routes (for example in food and/or water; subcutaneously, intraperitoneally, oral gavage (max. 2x daily, for 8 weeks), implanted with an osmotic pump (1x) or via intrathecal or intracerebroventriuclar (ICV) injection or via a cannula. These latter three procedures are done only once/mouse and require surgery with adequate general anesthesia and perioperative analgesia. As most drugs do not persist beyond 16 weeks, treatment will be for a max. of 20 weeks (max. 4 weeks preceding the testing and 16 weeks during testing). A subset of animals may receive perinatal drug treatment, which we will administer (IP, oral gavage or subcutaneous) to the pregnant or lactating dams. Another subset of animals may receive treatment in utero, for which the drug will be administrated to the pups by intraventricular injection, for which we need to perform surgery on the pregnant mothers. A pregnant dam will be operated aseptically under peri-operative analgesia and general anaesthesia at 16-19 days of timed pregnancy (third trimester), and the uterus is carefully exposed. Each of the foetuses will be injected with the drug in the lateral ventricles leaving the uterus intact. After this, the uterus is carefully placed back into the abdominal cavity and the abdominal incision will be closed by multiple layers of sutures. The total surgery time is maximally 30 min, from the moment the dam is anesthetised until she wakes up again, and 5-6 days later pups will be born naturally. The dam will be sacrificed after weaning without undergoing any other procedures. The animals are randomly selected for the respective drug treatments to be tested and the handler/observer (tester) is blinded for the specific genotype or treatment. All animals are humanely killed after testing to obtain tissue for analysis.The tests mentioned here are examples of behavioural assays that the mice may be subjected to. Which (groups of) tests largely depends on the mutation the mice carry and the phenotypes presented by the animal model. As an example, for Angelman Syndrome mouse models the mice are subjected to the accelerating Rotarod, open field, marble buying, nest building and 5.1 lid1c Which tests we will subject our mice to will be detailed and justified for each group of mice in future study plans.

-

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

The minimal group size needed to obtain a significant difference in a particular behavioural test depends on the mutant mouse line in question. As an example, below is an example of the group size needed for Angelman syndrome mice. From extensive experience gathered over the past years, we have determined that groups consisting of 15 mice per genotype are sufficient to show a robust difference between controls and Angelman syndrome mice (7). Using a meta-analysis (7) the statistical power of the subtests was determined. In table 2 (see below) is indicated how many mice are needed for each behavioural test. In our study a power of 0.80 will be used, considering some unexpected variation we will use n=15. This provides us with sufficient statistical power to run a 1- or 2-way ANOVA and detect meaningful effect sizes. We typically do not consider phenotypes to be robust if more than 15 animals are required. On the other hand, we do not go below 10 animals per group because it limits our statistical power and analysis, and becomes

too vulnerable for outliers. Experience has also helped us determine the number of mice need to run a well powered EEG experiment. We need 12 mice per genotype to detect meaningful effect sizes for each dose of therapeutic.For each new mutant mouse model, the tasks that will be employed will be based on what is known in the literature or expected, based on the nature of the mutation and gene involved. The presence of a performance difference between WT and mutant mice will first need to be verified for each individual task, and the robustness of the performance difference will determine the group size.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice	N/A (Annex I species specifically bred)	Embryo's, pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	Mus musculus: genetically modified and wild type littermates in different standard genetic backgrounds (eg Bl6, 129) or combinations (eg F1 Bl6 and 129) thereof. We choose this species based on the fact that they are genetically similar to humans and because we are able to test relevant parameters (eg. motor learning, cognitive tests, epilepsy etc) in mice that are often translatable to humans
Origin	All animals are bred in the institute or come from a registered supplier ensuring the animals are genetically pure and free of known infectious agents. The mice are socially housed, whenever possible, under the same standard conditions unless the behavioural test does not allow for this (eg. Nest building test). In those situations, the mice are single-housed for as short a period as possible.
Life stages	For behavioural or EEG analyses, embryos (E16-19; Requires surgery of mothers) or neonatal pups may be treated with the therapeutic of interest. The actual behavioural testing or placement of the neuro-logger will commence at ages 3–6 weeks. When juvenile, adolescent or adult (3, 6 or 8 week old respectively) mice are used, behavioural testing or neuro-logger placement will start 3-6 weeks after administration of therapeutic. This allows us to properly study the different developmental stages of the mice. The chosen age will depend on the functional test and whether the age is suitable for the test. Most typical is however 6-14 weeks.
Number	As explained earlier in the "experimental approach", from extensive experience gathered over the past 15 years, we have determined that for a typical behavioural test we need a group size of (max) 15 WT and 15 mutant mice. This provides us with sufficient statistical power to run a 1 or 2-way ANOVA and detect meaningful effect sizes. Smaller effect sizes

can be detected by larger numbers, but the biological relevance of such small effects is often questioned. Conversely, given the variability of behavioural testing, smaller group sizes can readily result in Type I or Type II errors. For Cre lines as well for therapeutic/pharmacological interventions, we will need 4 genotypes/groups (WT vehicle, WT treated, mutant vehicle and mutant treated).

Below we estimate the maximum number of mice we will use. For each experiment the number of mice will be detailed and justified in the study plan.

We estimate that for the coming 5 years we will need maximally:

Aim 1: Investigate the role of (nuclear) UBE3A in brain development.

We estimate that we will test 5 lines. These mice will be subjected to:

5.1 lid1c

B) Cognitive/social deficits/audiogenic seizures: 5 5.1 lid1c

Aim 2: Study the role of UBE3A in the cytosol/synapse

We estimate that we will test 5 lines. These mice will be subjected to:

5.1 lid1c

B) Cognitive/social deficits/audiogenic seizures: 5.1 lid1c

Aim 3: Study the effect of neighbouring genes in the 15q11-13 gene cluster

We estimate that we will test 10 lines. These mice will be subjected to:

5.1 lid1c

B) Cognitive/social deficits/audiogenic seizures: 5.1 lid1c

5.1 lid1c

Aim 4: Identify brain regions that are affected by loss of UBE3A and the phenotypes to which these brain regions contribute.

5.1 lid1c

We estimate that we will test 10 lines. These mice will be subjected to:

5.1 lid1c

B) Cognitive/social deficits/audiogenic seizures: 5.1 lid1c

Aim 5: 5.1 lid1c

	Treatment >P21 5.1 lid1c B) Cognitive/social deficits/audiogenic seizures: 5.1 lid1c 5.1 lid1c Treatment P1 For these mice we will treat mice within 24hrs after birth. 5.1 lid1c B) Cognitive/social deficits/audiogenic seizures: 5.1 lid1c Treatment embryo's In exceptional cases, treatment must occur in utero (eg. early treatment window or for efficient viral transduction). For these mice the pregnant dams are operated on and the embryos get an ICV injection with the therapeutic at E14-18. The pups are born normally after surgery and are followed thereafter. We estimate that this will only occur for 2 mouse lines. 5.1 lid1c Audiogenic seizure susceptibility assay: 5.1 lid1c
Gender	AS phenotypes do not differ between male and female patients and mice, and hence we will use both sexes. In the event there is a gender bias, we will choose the gender with the strongest phenotype.
Genetic alterations	Most GA animals will show no harmful phenotype, but maximally one newly generated GA line may show a mild harmful phenotype.The treatments described for the induction of the GA alleles and/or treatments with drugs/therapeutics will in some cases begin before birth. For this, it may be necessary to initiate treatment during the gestation period by treating the mothers. This will allow us to study the drug effects during development
Strain	Different standard genetic backgrounds (eg Bl6, 129) or combinations thereof (eg F1 Bl6 and 129) are used due to the sensitivity of the test paradigm to genetic background. For example the majority of our behavioural assays are carried out in a Bl6/129 background as a phenotypic difference is seen in these mice and not in pure Bl6 mice.

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

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

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

The discomfort depends on how the therapeutic is administered. This can be through IP, SC or IV injections without the use of anaesthesia. When mice are injected ICV, intracerebrally, via cannula or pump, inhalation anaesthesia (P>20) + analgesic will be used. For P0-P3 ICV or intracerebral injections, hypothermia-anaesthesia is used

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

Whenever possible, mice are group-housed but some tasks, such as the nest building task, requires the mice to be single housed. The order of tasks will be planned in such a way as to minimise the length of time the mice are single housed.

For tasks that require food restriction, such as with 5.1 lid1c , mice will be maintained at 85% of their ad libitum weight.

With the exception of a few tasks, we do not expect the procedures to compromise the welfare of the animal beyond the distress resulting from the test. However, some of the animals are treated with a therapeutic. These compounds could potentially have unforeseen side effects. Also, it is possible that the animals do not recover well from surgery after intraventricular injection, implantation of a mini pump, or from oral gavage or injection of the therapeutic. Most GA animals will not have a harmful phenotype, but maximally 2 GA lines may show a mild harmful phenotype.

Explain why these effects may emerge.

This is expected to be a rare situation since the impacts of most procedures are well known, however, the therapeutics implemented are new and may elicit side effects.

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

If unexpected adverse effects are observed, immediate action will be taken. E.g., the drug dose will be reduced or treatment may be withdrawn altogether. If appropriate we will administrate pain medication or remove the animal from the experiment and euthanise it.

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:
1. The animal stops eating or drinking.
2. Decrease of body weight of 10% relative to their weight at the start of the experiment, stunted growth before onset of the experiment (15% reduced compared to littermates).
3. For the 5.1 lid1c task, when a reduction in body weight of 20% or more occurs, the animals are removed from the experiment and fed ad libitum. If bodyweight decreases further the animals will be humanely killed.
4. Moderate circulatory or respiratory problems.
5. The development of a clinical neurological disease that results in sustained suffering (eg they display deviant behaviour such as abnormal aggression, epilepsy, lethargy).

Indicate the likely incidence.

According to our extensive experience with GA lines in the last 20 years, it is very rare to observe these events. We estimate this to be less than 10%.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

5.1 lid1c

Figure 3: Summary of estimated discomfort scores

P1 pups: Depending on the therapeutic it may be limited to a single IP, IV, IM or SC injection. Often though, P1 pups receive ICV or intracerebral injections using hypothermia-anaesthesia. The mice will subsequently be subjected to behavioural scrutiny or have a electrodes/pedestal placed at >P21 to enable brain activity measurements (Severity 3, Moderate)

>P21 mice: Also here therapeutic may be delivered IP, IV, IM or SC. However, delivery can also occur via ICV, pump or cannula, in some cases coinciding with the placement of electrodes/pedestal, all done under general anaesthesia. (severity 3, moderate) Pregnant/lactating dams (no treatment): These are the mothers of the P1 pups above, that serve as mothers for the pups (see P1 pups above) until P21. These dams are NOT treated (severity 2, mild).

Embryos: A limited number of embryos will be treated in utero (ICV injection) and placed back into the mom. (Severity 3, moderate).Pregnant/lactating dams of treated embryos: The mothers of the aforementioned embryos will be subjected to abdominal surgery under general anaesthesia to enable the treatment of the embryos (Severity 3, moderate)

For some new lines, it may involve breeding with a mild harmful phenotype but that is difficult to predict (Severity 2, mild).

G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to study the effect of the genetic modification on brain development and brain function. To date, studying GA mice is the only reliable method to investigate the function of a gene in brain development and brain function *in vivo*.
Reduction	All therapeutics to be studied are all first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when the therapeutic is found to function well in in vitro and ex-vivo experiments, will it be tested in vivo, which reduces the number of animals we use.
Refinement	The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of Angelman syndrome, and participate in scientific meetings. Also we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

All mice will be killed at the end of the experiment to allow for tissue isolation and analysis.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia. This causes least pain and stress for the animals

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons or to resolve welfare concerns

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 4 Type of animal procedure: 5.1 lid1c

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.

After testing the efficacy of potential therapeutics on 5.1 lid1c the therapeutic needs to be tested *in vivo*. Making mouse models for each individual patient mutation is impractical and wasteful in terms of number of animals.

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

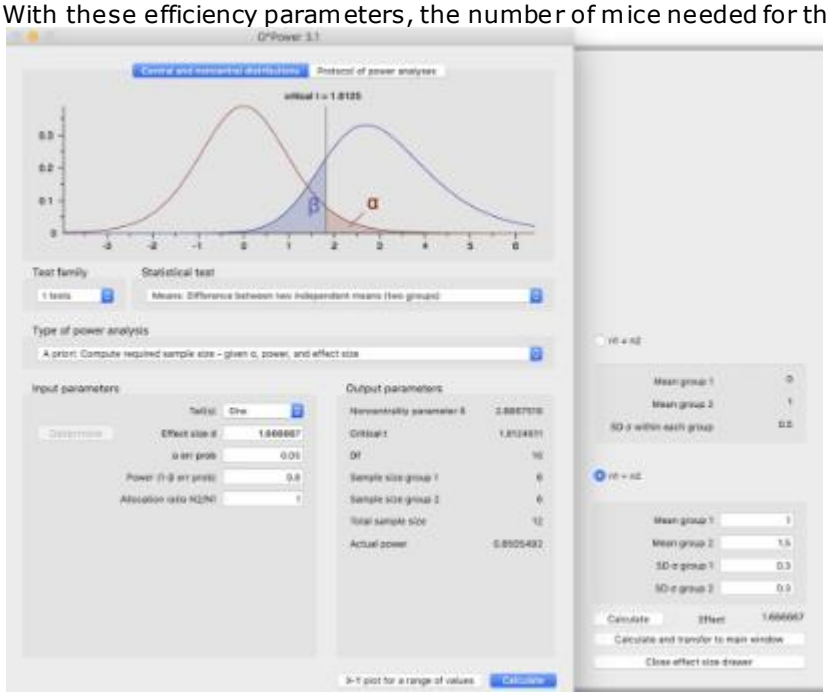
5.1 lid1c Treatment will be alone or in combination (one treatment regimen per mouse), continuously or intermittently by one or more routes (for example in food and/or water; subcutaneously, intraperitoneally, oral gavage (max. 2x daily, for 8 weeks), implanted with an

osmotic pump (1x) or via intrathecal or intracerebroventriuclar (ICV) injection or via a cannula. These latter three procedures, including the implantation of the cannula are done only once/mouse and require surgery with adequate general anesthesia and perioperative analgesia. As most drugs do not persist beyond 16 weeks, treatment will be for a max. of 20 weeks (max. 4 weeks preceding the testing and 16 weeks during testing).

All individual treatments impart a moderate discomfort to the animals, with the exception of the addition of therapeutic to drinking water or food. 5.1 lid1c

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

Based on previous experience, to be able to see a 1.5 fold change in expression levels with a SD of 0.3, using an alpha of 0.05 and beta of 0.8, we need 6 successfully xenografted pups per therapeutic treatment for each dosage in order to draw a conclusion. In our hands the survival rate of the pups after neonatal ICV injection is 80%. Also, the treatment with therapeutic 2-5 weeks post xenograft has a survival rate of 95%. With these efficiency parameters, the number of mice needed for the experiment is calculated.



B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice	N/A (Annex I species specifically bred)	Embryo's, pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	Mus musculus: genetically 5.1 lid1c

Origin	All animals are bred in the institute and are socially housed, whenever possible, under the same standard conditions. This ensures the animals are genetically pure and free of known infectious agents.
Life stages	Xenografts are performed on neonatal mice as at this age the mouse brain is still developing and allows the immature human cells to follow suit. Therapeutics are administered (via SC, IP, IV, IM, ICV injection, cannula, oral gavage or implanted osmotic pump) 2-5 weeks after transplantation to allow the mouse to recover and to allow the transplanted cells to mature and migrate out. Mice are sacrificed 1-6 weeks later.
Number	In the coming 5 years we expect to test 10 different 5.1 lid1c [redacted] mice/group. We estimate to test 5 variations per mutation (therapeutic, dose and time points), and 5 parameters. 5.1 lid1c [redacted] the number of mice needed for the experiment is calculated.
Gender	Both genders are used unless the mutation shows a clear gender bias. If there is a gender bias, all neonates will be treated (no gender determination possible at this age) and subsequent experiments will only be carried with the affected gender.
Genetic alterations	5.1 lid1c [redacted]
Strain	5.1 lid1c [redacted]

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

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

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

The neonates will receive hypothermia-anaesthesia. For the subsequent treatment with therapeutic or vehicle, if carried out by ICV the mice will receive general anaesthesia and peri-and postoperative analgesia.

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

We do not expect the procedures to compromise the welfare of the animal. However, some of the animals are treated with a therapeutic. These compounds could potentially have unforeseen side effects. Also, it is possible that the animals do not recover well from surgery after intraventricular or intracerebral injection of the cells, implantation of a mini pump, or from oral gavage or injection (ICV, intraperitoneal, intraventricular, intravenous or subcutaneous) of the therapeutic.

Explain why these effects may emerge.

This is expected to be a rare situation since the impacts of most procedures are well known, however, the therapeutics implemented are new and may elicit side effects.

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

If unexpected adverse effects are observed, immediate action will be taken. E.g., the drug dose will be reduced or treatment may be withdrawn altogether. If appropriate we will administrate pain medication or remove the animal from the experiment and euthanise it.

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:

1. The animal stops eating or drinking.
2. Decrease of body weight of 10% relative to their weight at the start of the experiment, stunted growth before onset of the experiment (15% reduced compared to littermates).
3. Moderate circulatory or respiratory problems.

Indicate the likely incidence.

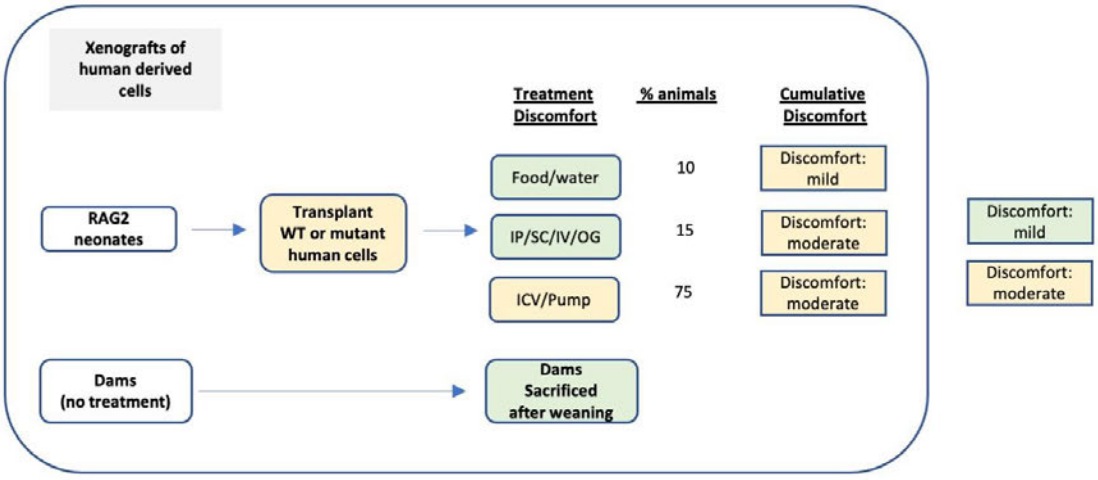
From our experience, very rarely do mice reach a HEP and need to be taken out of experiment. We estimate that this occurs in less than 10% of treated mice.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Severity	#	%	Treatment Groups
5.1 lid1c			

P1 pups: 5.1 lid1c under hypothermia-anaesthesia. Subsequently, the mice will receive a therapeutic (IP, IV, IM or SC injection) or via ICV requiring general anaesthesia (Severity: Moderate)
Pregnant/lactating dams (no treatment): These are the mothers of the P1 pups above, serving as mothers for the pups (see P1 pups above) unt il P21. These dams are NOT treated (severity: Mild)



G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to study the effect of the genetic modification on brain development and brain function in relevant cell-types and in an *in vivo* environment.
Reduction	Before we embark on choosing the genes for generating novel GA lines, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation. Once we decide to test a therapeutic, this is first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when the therapeutic is found to function well in in vitro and ex-vivo experiments, will it be tested in vivo, which reduces the number of animals we use. 5.1 lid1c
Refinement	The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of neurodevelopmental disorders, and participate in scientific meetings. Also we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

All mice will be killed at the end of the experiment to allow for tissue isolation and analysis.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia. This causes least pain and stress for the animals

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.
Animals are only killed for scientific reasons or to resolve welfare concerns.

NIET-TECHNISCHE PROJECTSAMENVATTING

7

Naam van het project	Mechanismen die ten grondslag liggen aan Angelman Syndroom
NTS-identificatiecode	NTS-NL-568710 v.1
Nationale identificatiecode van de NTS *Veld wordt niet gepubliceerd.*	
Land	Nederland
Taal	nl
Indiening bij EU *Veld wordt niet gepubliceerd.*	ja
Duur van het project, uitgedrukt in maanden.	60
Trefwoorden	Angelmen syndroom Muismodellen verstandelijk handicap RNA/DNA therapie
Doel(en) van het project	Fundamenteel onderzoek: Zenuwstelsel Omzettinggericht en toegepast onderzoek: Zenuwziekten en psychische aandoeningen van de mens

DOELSTELLINGEN EN VERWACHTE VOORDELEN VAN HET PROJECT

Beschrijf de doelstellingen van het project (bijvoorbeeld het aanpakken van bepaalde wetenschappelijke onduidelijkheden, of wetenschappelijke of klinische behoeften).	Angelman syndroom (AS) is een ernstige neurologische aandoening die bij ongeveer 1: 20.000 geboorten voorkomt. Het resulteert in een ernstige ontwikkelingsachterstand, verstandelijke handicap, motorische stoornissen, gedragsafwijkingen waaronder autisme en verstoorde slaapcyclus, en de afwezigheid van spraak. Een groot aantal patiënten (80%) heeft epileptische aanvallen. Angelman-syndroom wordt voornamelijk veroorzaakt door verlies van functioneel UBE3A-eiwit. Dit voorstel richt zich specifiek op het verkrijgen van inzicht in de functie van het UBE3A-eiwit in de hersenontwikkeling. We hebben onlangs ontdekt dat UBE3A is gericht op de celkern en hebben onthuld hoe het eiwit in de celkern komt, maar de functie ervan in de kern is volledig onbekend. Het UBE3A-gen bevindt zich in het 15q11-13-gebied op chromosoom 15, een gebied dat bij de meeste AS patiënten volledig is verwijderd. Hoewel we weten dat verlies van UBE3A voldoende is om het Angelman-syndroom te ontwikkelen, lijkt het erop dat de naburige genen ook kunnen bijdragen aan de aandoening, met name aan epilepsie. Daarom willen we meer inzicht krijgen in de rol van deze genen en onderzoeken of geneesmiddelen die de UBE3A-functie kunnen herstellen, ook therapeutisch voordeel hebben als het hele 15q11-13-gebied ontbreekt. 5.1 lid2h
Welke potentiële voordelen kan dit project opleveren? Leg uit hoe de wetenschap vooruit kan worden geholpen of mensen, dieren of het milieu uiteindelijk voordeel kunnen hebben bij het project. Maak, waar van toepassing, een onderscheid tussen voordelen op korte termijn (binnen de looptijd van het project) en voordelen op lange termijn (die mogelijk pas	We hopen dat ons onderzoek leidt tot een betere begrijp van hoe het UBE3A eiwit functioneert in samenwerking met eiwitten van omliggende genen. Dit kan leiden tot nieuwe behandelingen voor kinderen met Angelman syndroom. We hebben eerder laten zien dat we bevindingen in muizen kunnen vertalen naar de kliniek, zoals een genetische therapie voor de behandeling van kinderen met het Angelman-syndroom, die momenteel wordt uitgevoerd 5.1 lid2h

worden bereikt nadat het project is afgerond).	

VOORSPELDE SCHADE

In welke procedures worden de dieren gewoonlijk gebruikt (bijvoorbeeld injecties, chirurgische procedures)? Vermeld het aantal en de duur van deze procedures.	Appendix 1: Generatie en import nieuwe lijnen en fokken met ongerief 5.1 lid1c - Mannen worden via een vasectomie gesteriliseerd. 5.1 lid1c -Fokken van muizen met een mild fenotype als gevolg van de mutatie 5.1 lid1c Appendix 2: Verzamelen van muizenweefsel met en zonder behandeling -Opzetten van neuronale culturen met hersenen uit embryo's. Moeders worden verdoofd en gedood (aantal 5.1 lid1c en de embryo's geïsoleerd en gelijk gedood zonder behandeling en hersenweefsel gebruikt (aantal 5.1 lid1c 5.1 lid1c
Wat zijn de verwachte gevolgen/nadelige effecten voor de dieren, bijvoorbeeld pijn, gewichtsverlies, inactiviteit/verminderde mobiliteit, stress, abnormaal gedrag, en wat is de duur van die effecten?	Appendix 1: Generatie en import nieuwe lijnen en fokken met ongerief In het algemeen hebben de muizen niet al te veel last van de behandelingen. De operaties worden gedaan onder narcose en pijnstilling en zijn de dieren binnen een paar uur weer actief. Omdat we hier te maken hebben met genetisch gemodificeerde muizen is het mogelijk dat sommige nieuwe lijnen een mild fenotype vertonen. We schatten in dat maximaal 10% van de experiment muizen hier last van heeft. Dit kan variëren van een milde motorische afwijking tot epileptische aanvallen. Appendix 2: Muizenweefsel met en zonder behandeling Voor een aantal dieren (moeders en embryo's neuronale culturen) worden de muizen gedood zonder voorafgaande (be)handeling. De overige dieren worden behandeld via injecties of operatief behandeld onder narcose en postoperatieve pijnstilling. Appendix 3: Karakterisatie van behandelde of onbehandelde muizen. 5.1 lid1c Soms is individuele huisvesting nodig wat stressvol is voor de muizen. De duur hiervan wordt zo kort mogelijk gehouden. Ook voor bepaalde taken is het nodig om de dieren te onderwerpen aan voedseldeprivatie, om ze te motiveren om voedsel als beloning te zien. Een voorbeeld van een stressvolle taak is de audiogenic seizure test waar muizen gedurende maximaal 20 seconden blootgesteld worden aan ±100dB geluid. Bij sommige mutantmuizen lijnen leidt dit tot een epileptische aanval. Dit wordt een keer gedaan.

	Muizenlijnen waarvan verwacht wordt dat ze gevoelig zijn voor epileptische aanvallen worden voorzien van een (draadloos) EEG meetinstrument. Hiermee wordt de hersenactiviteit gemeten dmv electrodes die operatief geplaatst worden in aanwezigheid van postoperatieve pijnstilling. De hersenactiviteit wordt voor een periode van 2 weken bekeken. Appendix 4: Karakterisering van met menselijke iPSC afgeleide cellen in hersenen van behandelde of onbehandelde muizen. Na het inbrengen van menselijke cellen onder verdoving in pasgeboren pups, zien we dat de pups kort na terugplaatsing bij de moeder weer actief zijn. Ook bij het onder narcose toegediende geneesmiddel, worden de dieren een paar uur na de operatie weer actief.
Welke soorten en aantallen dieren zullen naar verwachting worden gebruikt? Wat zijn de verwachte ernstgraden en de aantallen dieren in elke ernstcategorie (per soort)?	

Soort:	*Totaal aantal*	*Geraamde aantallen naar ernstgraad*			
		Terminaal	*Licht*	*Matig*	*Ernstig*
Muizen (Mus musculus)	5.1 lid1c				

Wat gebeurt er met de dieren die aan het einde van de procedure in leven worden gehouden?			
Soort:	*Geraamd aantal te hergebruiken, in het habitat-/houderijsysteem terug te plaatsen of voor adoptie vrij te geven dieren*		
	Hergebruikt	*Teruggeplaatst*	*Geadopteerd*

Geef de redenen voor het geplande lot van de dieren na de procedure.	Dieren worden aan het eind van de experiment gedood en weefsels verzameld voor biochemische, immunocytochemische en electrofysiologische analyses.

TOEPASSING VAN DE DRIE V'S

1. Vervanging Beschrijf welke diervrije alternatieven op dit gebied voorhanden zijn en waarom zij niet voor het project kunnen worden gebruikt.	Om inzicht te krijgen in het effect van een therapie op de ontwikkeling en functie van de hersenen, is het essentieel om de therapie te testen op een muismodel waarin de zelfde DNA verandering aanwezig als de patiënt die we willen behandelen. Tot op heden is het bestuderen van genetisch gemodificeerde muizen de enige betrouwbare methode om de functie van een gen in hersenontwikkeling en hersenfunctie in levende dieren te onderzoeken.
2. Vermindering Leg uit hoe de aantallen dieren voor dit project zijn bepaald. Beschrijf de stappen die zijn genomen om het aantal te gebruiken dieren te verminderen en de beginselen die zijn gebruikt bij het opzetten van de studies. Beschrijf, waar van toepassing, de praktijken die gedurende het hele project zullen worden toegepast om het aantal dieren die in overeenstemming met de wetenschappelijke doelstellingen werden gebruikt, tot een minimum te beperken. Deze praktijken kunnen bijvoorbeeld bestaan uit proefprojecten, computermodellen, het delen van weefsel en hergebruik.	In het huidige PLA worden alle te onderzoeken therapieën eerst uitgebreid in gekweekte cellen getest, en pas als de therapie goed blijkt te werken in deze cellen, zal het in proefdieren worden getest, wat het aantal dieren dat we gebruiken vermindert. Het maximaal benodigde dieren per experiment wordt met name bepaald op basis van eerdere experimenten. Om die aantallen laag te houden maken we daarbij zo veel mogelijk gebruik van testen die een maximaal verschil laten zien tussen behandelde en controledieren. Ook realiseren we een vermindering van de benodigde dieren door: - zowel mannen als vrouwen te gebruiken wanneer de mutatie dit toelaat. -De volgorde waarin testen met hetzelfde dier worden uitgevoerd geeft ons de meeste informatie met het minst aantal dieren. -Gebruik van geoptimaliseerde protocollen om de gevoeligheid te maximaliseren met een minimale groepsgrootte. -We maken gebruik van goed gedefinieerde en gestandaardiseerde genetische achtergronden.
3. Verfijning Geef voorbeelden van de specifieke maatregelen (bv. verscherpte monitoring, postoperatieve behandeling, pijnbestrijding, training van dieren) die in verband met de procedures moeten worden genomen om de welzijnskosten (schade) voor de dieren tot een minimum te beperken. Beschrijf de mechanismen om gedurende de looptijd van het project nieuwe verfijningstechnieken in gebruik te nemen.	Om stress verschijnselen te minimaliseren bij onze muizen tijdens gedragstesten worden ze 1) zo veel mogelijk groep-gehuisvest 2) dagelijks 'gehandeld' om de dieren aan de onderzoekers gewend te laten raken. De onderzoekers blijven op de hoogte van de laatste ontwikkelingen op dit onderzoeksgebied door (inter)nationale samenwerking met andere onderzoeksgroepen die onderzoek doen naar de genetische achtergrond van neurologische ontwikkelingsstoornissen, en nemen deel aan wetenschappelijke bijeenkomsten. Ook controleren we door middel van uitgebreide literatuuronderzoeken (Medline, Pubmed) of er geen geschikte alternatieven beschikbaar zijn voor een bepaald project. Tijdens ons lopende onderzoek zal intensief literatuuronderzoek worden voortgezet op zoek naar nieuwe ontwikkelingen met betrekking tot alternatieve benaderingen die in deze studie moeten worden geïmplementeerd.
Licht de keuze van de soorten en de bijbehorende levensstadia toe	Muizen in alle stadia (vanaf embryo, pasgeborenen tot volwassenen) zullen worden onderworpen aan verschillende analyses zonder of na behandeling met een geneesmiddel. Hierdoor kunnen we de verschillende ontwikkelingsstadia van de hersenen goed bestuderen. De gekozen leeftijd hangt af van de functionele test en of de leeftijd geschikt is voor die test. De beschreven behandelingen met medicijnen zullen in sommige gevallen al voor de geboorte beginnen, om de effecten op de embryo's tijdens de ontwikkeling te bestuderen. 5.1 lid1c

	5.1 lid1c

VOOR EEN BEOORDELING ACHTERAF GESELECTEERD PROJECT

Project geselecteerd voor BA?	nee
Termijn voor BA	
Reden voor de beoordeling achteraf	
Bevat ernstige procedures	
Maakt gebruik van niet-menselijke primaten	
Andere reden	
Toelichting van de andere reden voor de beoordeling achteraf	

AANVULLENDE VELDEN

Nationaal veld 1 *Veld wordt niet gepubliceerd.*	
Nationaal veld 2 *Veld wordt niet gepubliceerd.*	
Nationaal veld 3 *Veld wordt niet gepubliceerd.*	
Nationaal veld 4 *Veld wordt niet gepubliceerd.*	
Nationaal veld 5 *Veld wordt niet gepubliceerd.*	
Startdatum project *Veld wordt niet gepubliceerd.*	
Einddatum project *Veld wordt niet gepubliceerd.*	
Goedkeuringsdatum project *Veld wordt niet gepubliceerd.*	
ICD-code 1 *Veld wordt niet gepubliceerd.*	
ICD-code 2 *Veld wordt niet gepubliceerd.*	
ICD-code 3 *Veld wordt niet gepubliceerd.*	
Link naar de eerdere versie van de NTS buiten het EC-systeem	

Van: info@zbo-ccd.nl
Verzonden: maandag 22 augustus 2022 11:12
Aan: 5.1 lid2h
Onderwerp: Verzoek om advies over projectvergunningsaanvraag AVD5.1 lid2h202216352
Bijlagen: 5.1 lid2hAVD5.1 lid2hxxxx _2022_0013__Project proposal.docx; 5.1 lid2hAVD5.1 lid2hxxxx _2022_0013__Appendix 2_DEC.docx; 5.1 lid2hAVD5.1 lid2hxxxx _2022_0013__NTS_DEC.xlsx; 5.1 lid2hAVD5.1 lid2hxxxx _2022_0013__Appendix 4_DEC.docx; 5.1 lid2hAVD5.1 lid2hxxxx _2022_0013__Appendix 1_DEC.docx; 5.1 lid2hAVD5.1 lid2hxxxx _2022_0013__Aanvraag Projectvergunning.pdf; 5.1 lid2hAVD5.1 lid2hxxxx _2022_0013__Appendix 3_DEC.docx

Categorieën: Nieuwe aanvragen (of nummer)

Geachte leden van 5.1 lid2h

De Centrale Commissie Dierproeven (hierna: CCD) verzoekt u in het kader van vergunningverlening advies te geven over het project met als titel: "Mechanisms underlying the pathophysiology of Angelman Syndrome" en aanvraagnummer: AVD5.1 lid2h202216352.

Uw commissie wordt verzocht op grond van artikel 10.a.2 van de Wet op de dierproeven de aanvraag te beoordelen en een ethische toetsing uit te voeren waarbij wordt afgewogen of de doelstelling van het project, de verwachte voordelen voor mens, dier of milieu en de haalbaarheid van de doelstellingen, het gebruik van dieren en de schade die zal worden toegebracht aan de dieren in de vorm van lijden, pijn en angst kan rechtvaardigen.

Graag ontvangen wij van u bericht dat deze e-mail goed is ontvangen en wanneer u dit advies in de vergadering gaat bespreken.

Voor het in te dienen advies dient de DEC gebruik te maken van de meest actuele versie van het op de website van de CCD gepubliceerde Format DEC-advies en de toelichting daarbij. U dient deze aanvraag vertrouwelijk te behandelen. Voor de communicatie met de CCD dient u gebruik te maken van FileSecure.

De CCD verzoekt u uiterlijk binnen 20 werkdagen, na 22-08-2022, uw advies bij de CCD in te dienen. Indien de aanvraag door uw commissie niet in behandeling kan worden genomen, dient u dit per ommegaande per e-mail aan de CCD te melden.

Ingeval uw commissie tussentijds aanvullende informatie wil inwinnen bij de aanvrager wordt de termijn opgeschort en geeft u in uw advies aan wanneer dit is geweest. Opschorting van de adviestermijn vindt niet plaats ingeval u ten behoeve van uw advies een onafhankelijk extern expert raadpleegt. Mocht u verwachten door een andere reden dan opschorting uw advies later dan 20 werkdagen na 22-08-2022 bij de CCD in te dienen, dan verzoeken wij u dit direct aan de CCD te melden.

Mocht u vragen hebben, dan kunt u uiteraard contact met ons opnemen.

Met vriendelijke groet,
Centrale Commissie Dierproeven

www.centralecommissiedierproeven.nl

.....

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.....
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Format DEC-advies

Maak bij de toepassing van dit format gebruik van de Praktische Handreiking: Ethisch Toetsingskader voor proefdiergebruik. Voor voorbeelden, zie bijlage I.

Herhaling van antwoorden is niet nodig. Indien van toepassing kan verwezen worden naar een bij een eerdere vraag verstrekt antwoord.

A. Algemene gegevens over de procedure

1. Aanvraagnummer AVD5.1 lid2h202216352

2. Titel van het project: Mechanisms underlying the pathophysiology of Angelman and Dup15q syndrome.

3. Titel van de NTS: Mechanismen die ten grondslag liggen aan Angelman Syndroom

4. Type aanvraag:
   nieuwe aanvraag projectvergunning

5. Contactgegevens DEC:
   - naam DEC: DEC van 5.1 lid2h
   - telefoonnummer contactpersoon: 5.1 lid2e
   - e-mailadres contactpersoon:5.1 lid2h

6. Adviestraject (data dd-mm-jjjj):
   ontvangen door DEC:02-08-2022 (verzoek om advies via CCD:22-08-2022)
   aanvraag compleet: 20-01-2023
   in vergadering besproken : 22-08-2022, 31-10-2022, 29-12-2022
   anderszins behandeld: schriftelijke ronde kleine commissie: 20-01-2023
   termijnonderbreking(en) van 26-08-2022 tot 24-10-2022
   van 08-11-2022 tot 01-12-2022
   van 04-01-2023 tot 20-01-2023
   besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   aanpassing aanvraag : 24-10-2022, 01-12-2022 en 20-01-2023
   advies aan CCD en aanvrager: 13-02-2023

7. De aanvraag is afgestemd met de IvD van de instelling, een vertegenwoordiger van de IvD woonde de besprekingen bij voor toelichting.

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

9. Correspondentie met de aanvrager
   - Datum : 26-08-2022 (correspondentie 1)
   - Gestelde vragen betroffen de volgende onderwerpen:
     Project
     - Referentie naar niet aanwezige appendix
     - Is er niet sprake van categorie 'Basic research'
     - Aansluiting doelstellingen bij inleiding
     - Rol van onderzoek naar therapeutische interventies nader toelichten/onderbouwen (incl. IPSc strategie)
     - Stroomschema niet duidelijk en sluit niet aan bij beschrijving in project

Appendices
- Tekstuele aanpassingen (verwarrend woordgebruik)
- Kaders 'Breeding of GA-lines with a harmful phenotype'. Aantallen dieren voor fok en aantallen dieren voor experiment. Aantallen dieren/aantallen lijnen
- Aantallen dieren sluiten op verschillende plaatsen niet altijd op elkaar aan
- Toelichting '*Animals are only killed for scientific reasons'*
- Entreecriteria voor appendix 2 experimenten
- Toelichten/onderbouwen maximum scenario m.b.t. ongerief
- Onderscheid voorbehandeling en therapeutische interventies
- Onderbouwing experimenten waarbij dieren met kop vastzitten
- Entreecriteria/overwegingen voor starten met extra- of intracraniale afleidingen
- Onderbouwing keuze (maximale) groepsgrootte, aantallen lijnen, aantallen interventies, aantallen dieren
- Aantal operaties op zwangere dieren
- Condities voor toevoegen van extra testen (max aantal testen per dier, maximaal ongerief)
- Onderbouwing voor opnemen appendix 4 in dit project. Entreecriteria voor starten experimenten, voor de te testen *'potential therapeutics', onderzoek met IPsc's*

NTS
- Bijstellen op basis van bijstellingen in project

- Datum antwoord: 24-10-2022
- De antwoorden hebben geleid tot een ingrijpende aanpassing van de aanvraag (alle experimenten gericht op therapeutische toepassingen zijn verwijderd).

- Datum : 08-11-2022 (correspondentie 2)
- Gestelde vragen betroffen de volgende onderwerpen:

Project
- Categorie 'Basic research'. Beschrijving/onderbouwing van wetenschappelijk belang en maatschappelijk belang (incl. beschrijving stakeholders)
- Aansluiting stroomschema bij beschrijving in project

Appendices
- Tekstuele aanpassingen (verwarrend woordgebruik)
- Kaders 'Breeding of GA-lines with a harmful phenotype' Beslismomenten/criteria voor als er dieren met aangetast fenotype worden geboren
- Ongerief supergeovuleerde vrouwtjes
- Toelichten/onderbouwen maximum scenario m.b.t. ongerief
- Toelichten/onderbouwen ICV toedieningen (in jonge en oudere dieren)
- Onderbouwing keuze aantallen lijnen, groepsgroottes, keuze parameters bij de verschillende type experimenten, statistische onderbouwing aantallen dieren (inclusief omgaan met dieren uit zelfde of verschillende nesten)
- Na verwijderen van therapeutische experimenten zijn nog enkele 'relicten' blijven staan
- Ongerief tgv bereiken humaan eindpunt en cumulatief ongerief tgv interventies niet herleidbaar
- Entreecriteria voor starten appendix 3 experimenten
- Experimenten uitgevoerd in lichtperiode? Is hierdoor sprake van additioneel ongerief?
- Nadere toelichting/onderbouwing van de uitvoering van de '*highly standardized behavior test battery'*
- Nadere toelichting/onderbouwing van situatie '*For novel mouse models'*
- Nadere toelichting/onderbouwing van '*Therapeutic rescues'*

- Overwegingen bij implementeren van een nieuwe test
- Situaties van afwijkende huisvesting
- Onderbouwing cumulatieve ongeriefinschattingen bij meerdere testen.

NTS

- Ontbreekt

- Datum antwoord: 01-12-2022
- De antwoorden hebben geleid tot ingrijpende aanpassing van de aanvraag.

- Datum : 04-01-2023 (correspondentie 3)
- Gestelde vragen betroffen in alle gevallen een nadere toelichting op antwoorden uit de vorige vragenronde:

Appendices

- Tekstuele aanpassingen (vooral nu niet meer relevante relicten blijven staan uit vorige versie)
- Kaders 'Breeding of GA-lines with a harmful phenotype' Beslismomenten/criteria voor als er dieren met aangetast fenotype worden geboren
- Onderbouwing keuze aantallen lijnen, groepsgroottes, keuze parameters bij de verschillende type experimenten, statistische onderbouwing aantallen dieren (inclusief omgaan met dieren uit zelfde of verschillende nesten)
- Experimenten uitgevoerd in lichtperiode? Is hierdoor sprake van additioneel ongerief?

NTS

- Bevat veel jargon en spelfouten

- Datum antwoord: tot 20-01-2023
- 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 projectvergunning

3. De DEC is competent om hierover te adviseren.

4. Geen van de DEC-leden is betrokken bij het project en de indiener en derhalve uitgesloten van de behandeling van de aanvraag en het opstellen van het advies.

C. Beoordeling (inhoud)

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft *(Zie handreiking 'Invulling definitie project'; zie bijlage I voor toelichting en voorbeeld).*

Deze aanvraag betreft primair fundamenteel onderzoek. Het beschreven onderzoek is een vervolg op een eerdere aanvraag (AVD5.1 lid2h) en richt zich op de effecten van genetische modificaties in het UBE3A gen. Dergelijke modificaties zijn bij mensen aanleiding tot ernstige neuronale ontwikkelingsstoornissen (intellectuele afwijkingen, motorische disfunctie en gedragsafwijkingen (autisme, slaap- en spraakstoornissen).

Meer specifiek richt het project zich op de biologie achter de pathofysiologie bij Angelman syndroom (AS, het verlies van het UB3A gen) en het Dup15q syndroom (waarbij sprake is van duplicatie van een aantal genen, inclusief het UBE3A gen). Het is de verwachting dat de in dit project voorgestelde experimenten naast fundamenteel wetenschappelijke kennis over de regulatie en de rol van het UB3A gen tijdens de hersenontwikkeling ook handvatten zou kunnen opleveren voor mogelijke therapeutische interventies. Er zijn op dit moment geen effectieve behandelingen voor deze ziektes beschikbaar.
Onder het vorige project zijn onder andere een serie gedragstesten ontwikkeld waarin 'AS muizen' een robuust fenotype laten zien. Het is de verwachting dat door het hierin testen van verschillende muismutanten en effecten van '*tool compounds'* kennis over de rol van UBE3A en aanknopingspunten voor mogelijke interventies kunnen worden geïdentificeerd.

Gedurende de behandeling van dit project zijn de doelstellingen en de strategie na de opeenvolgende bespreekrondes steeds (ingrijpend) bijgesteld.
Het feit dat het primair fundamenteel wetenschappelijk onderzoek betreft betekent dat er een zekere mate van onzekerheid is over de keuze en de uitvoering van de experimenten gedurende de looptijd van dit project.
Binnen deze context is de commissie van mening dat het in dit project gaat om een werkprogramma met welomschreven doelen en een logisch samenhangend geheel van (sub)doelstellingen dat één of meerdere dierproeven omvat. De vertaling van de doelstellingen naar de experimenten is op een acceptabel aggregatieniveau inzichtelijk en herleidbaar (zie ook de antwoorden op de vragen C15 en E3).
Voor de uiteindelijke uitwerking hiervan is de commissie mede uitgegaan van de ervaring en kennis van de wetenschappelijke infrastructuur van de betreffende onderzoeksgroep. Het is duidelijk gemaximeerd welke handelingen individuele dieren zullen ondergaan. Hieruit kan afgeleid worden met welk ongerief individuele dieren maximaal zullen worden geconfronteerd.
De commissie is ervan overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over het starten van dierproeven, over de opzet en voortgang hiervan en over het hierbij niet onnodig gebruik van dieren.
Gezien bovenstaande is de DEC van mening dat hier sprake is van een juiste invulling van de definitie project en dat de aanvraag voldoende samenhang heeft en toetsbaar is.

2. Signaleer of er mogelijk tegenstrijdige wetgeving is die het uitvoeren van de proef in de weg zou kunnen staan. Het gaat hier om wetgeving die gericht is op de gezondheid en welzijn van het dier of het voortbestaan van de soort (bijvoorbeeld Wet dieren en Wet Natuurbescherming).

   Er is geen tegenstrijdige wetgeving die het uitvoeren van de dierproeven in de weg zou kunnen staan.

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

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

Belangen en waarden

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een directe en reële relatie is tussen beide doelstellingen. Beoordeel of het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld *(Zie Praktische handreiking ETK: Stap 1.C4; zie bijlage I voor voorbeeld)*.

   Het uiteindelijke doel is het vergaren van kennis over de rol van het UBE3A gen in

de ontwikkeling van de hersenen en het op basis van deze kennis beschikbaar krijgen over aangrijpingspunten voor mogelijke gerichte behandelingen voor Angelman en Dup15q syndroom patiënten.

De directe doelen van dit project zijn het verkrijgen van inzicht in:

1. De rol van het UBE3A gen in de ontwikkeling van de hersenen.
2. De synergie tussen het UBE3A gen en de omringende genen in het de 15q11-13 gencluster in zowel AS als Du15q.
3. De hersendelen die aangedaan zijn door het verlies van het UBE3A gen.

De commissie is van mening dat de aanvrager, basis van de resultaten verkregen onder het voorgaande project en de inbedding van het voorgestelde onderzoek binnen de instelling en de verwijzing naar relevante wetenschappelijke literatuur, voldoende duidelijk heeft gemaakt dat het directe doel gerechtvaardigd is in de context van dit onderzoeksveld en de achterliggende klinische problematiek.

5. Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden welke morele waarden in het geding zijn of bevorderd worden (Zie Praktische handreiking ETK: Stap *2.B en tabel 1; zie bijlage I voor voorbeeld)*

De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de doelgroep/patiënten/maatschappij, de wetenschap (het onderzoeksveld), en de onderzoekers.

- Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De integriteit wordt aangetast door het instrumentele gebruik, de genetische modificatie, de huisvesting in een proefdierfaciliteit (beperkingen in hun fysiologische en ethologische behoeften), het geconfronteerd worden met 'ziekte' en het uiteindelijk gedood worden. Het welzijn van de dieren wordt aangetast door de chirurgische ingrepen, het door de genetische modificaties mogelijk optreden van epileptische aanvallen, het uitvoeren van de gedragstesten en de behandeling met verschillende stoffen. De dieren hebben er belang bij gevrijwaard te blijven van deze aantasting van hun integriteit en ongerief.
- Voor de patiënten en de samenleving (en dan primair de directe familie van de AS en Dup15q patiënten) zijn deze wetenschappelijke inzichten uiteindelijk van belang, omdat ze zouden kunnen bijdragen aan het beschikbaar komen van behandelingen voor deze ernstige ziektebeelden. Voor de patiënten is er ook een (beperkt) negatief belang. Bij hen wordt bloed afgenomen voor het genereren van IPSc afgeleide cellen.
- Voor de wetenschap en de onderzoekers is het beschikbaar komen van kennis over de rol van het UBE3A gen en de 15q11-13 gencluster bij de normale en afwijkende hersenontwikkeling van significant belang. Verder geldt naast dit wetenschappelijk belang dat het publiceren van belangrijke nieuwe wetenschappelijke inzichten een bijdrage levert aan hun wetenschappelijke reputatie en status, wat vaak de sleutel is voor het verkrijgen van nieuwe onderzoeksmiddelen en mogelijkheden. Carrièremogelijkheden en status kunnen door de onderzoekers zelf van belang geacht worden, maar dienen naar de mening van de DEC maar heel beperkt een 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 zorgprofessionals is kennis over de rol van het UBE3A gen en de 15q11-13 gencluster bij de normale en afwijkende hersenontwikkeling van belang met het oog op voorlichting van de patiënt en mogelijke toekomstige behandelmogelijkheden.

6. Is er aanleiding voor de DEC om de in de aanvraag beschreven effecten op het milieu in twijfel te trekken?

   In de aanvraag is aangegeven dat er geen effecten op het milieu worden verwacht. De DEC meent ook dat door de aangegeven barrière maatregelen (D1) die niet aanwezig zijn.

Proefopzet en haalbaarheid

7. Beoordeel of de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven voldoende gewaarborgd zijn. Licht uw beoordeling toe. *(Zie Praktische handreiking ETK: Stap 1.C5).*
   De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd. De commissie is overtuigd van de kwaliteit van het werk en de kennis van de aanvrager, zoals ook blijkt uit de in de aanvraag vermelde publicaties van de onderzoeksgroep. De commissie is ervan overtuigd dat de ervaring en expertise bij de aanvragende instantie er toe zal leiden dat de doelstellingen haalbaar zijn, dat er zorgvuldig met de proefdieren gewerkt zal worden en dat er niet onnodig dieren gebruikt zullen worden.
   Het onderzoek is ingebed in een brede klinische setting met veel kennis over en ervaring met de betreffende ziekte beelden
   De commissie heeft al jarenlang ervaring met het beoordelen van dit type experimenten bij deze vergunninghouder.

8. Beoordeel of het project goed is opgezet, de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstellingen en of de gekozen strategie en experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project. Licht uw beoordeling toe. *Zie Praktische handreiking ETK: Stap 1.C6)*.

   De directe doelstellingen van het project zijn realistisch en vanwege het overwegend fundamenteel wetenschappelijke karakter van het onderzoek sluiten deze op een hoger aggregatieniveau aan bij de voorgestelde experimentele opzet en uitkomstparameters (zie ook C1 en C4). De inherente onzekerheid in het fundamenteel wetenschappelijke onderzoek maakt een volledig onderbouwde strategie tot op het niveau van de uitvoering van elk individueel experiment gedurende de gehele looptijd van dit project onmogelijk. De indieners hebben duidelijk aangegeven welke zekerheden en onzekerheden hierbij een rol spelen. Tussen de verschillende stappen zijn go/no-go beslismomenten gedefinieerd.
   De DEC is van mening dat het project binnen deze context goed is opgezet, logisch aansluit bij de resultaten verkregen onder het voorgaande project en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de beschreven doelstellingen.

Welzijn dieren

9. Geef aan of er sprake is van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren. Beoordeel of de keuze hiervoor voldoende wetenschappelijk is onderbouwd en of de aanvrager voldoet aan de in de

Wet op de Dierproeven (Wod). voor de desbetreffende categorie genoemde beperkende voorwaarden. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C1; zie bijlage I voor toelichting en voorbeelden).*

Er is geen sprake van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren.

10. Geef aan of de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU. Indien niet aan deze minimale eisen kan worden voldaan, omdat het, om redenen van dierenwelzijn of diergezondheid of om wetenschappelijke redenen, noodzakelijk is hiervan af te wijken, beoordeel of dit in voldoende mate is onderbouwd. Licht uw beoordeling toe.

In principe zullen alle dieren conform de eisen in bijlage III van richtlijn 2010/63/EU worden gehuisvest. De studs en de gevasectomeerde mannelijke dieren worden individueel gehuisvest. In het kader van de wetenschappelijke experimenten zullen dieren alleen voorafgaand en gedurende een aantal gedragsexperimenten (de nestbouwtest, tijdens de voerbeperkingsperiode voorafgaand aan de cognitietesten) voor enige tijd individueel gehuisvest worden. Deze huisvesting is niet conform de richtlijn, maar is vanuit het belang van het experiment voldoende onderbouwd en in termen van ongerief geclassificeerd.

De commissie acht het uitvoeren van de gedragstesten in de lichtperiode ook een afwijking van de standaard huisvestingomstandigheden (zie ook C11).

11. Beoordeel of het cumulatieve ongerief als gevolg van de dierproeven voor elk dier realistisch is ingeschat en geclassificeerd. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C2)*.

Bij het genereren van nieuwe lijnen is er bij de donordieren (superovulatie), de gevasectomeerde mannen en de foster dieren sprake van cumulatief matig ongerief.
Gezien de aard van de betrokken genen is het niet uitgesloten dat er bij de genetisch gemodificeerde dieren sprake zou kunnen zijn van een lijn met constitutioneel ongerief. Bij de lijnen die tot nu toe gegenereerd zijn is dit nog nooit aangetroffen. Desondanks wordt de optie open gehouden dat er bij maximaal 1 lijn voor de fok en in de experimenten gebruik gemaakt zal worden van dieren met constitutioneel maximaal gering ongerief. Dieren met een fenotype resulterend in meer dan gering ongerief (de kans hierop is zeer klein) worden direct gedood.
Ten gevolge van de toedieningen en mogelijke effecten van de toegediende stoffen (voor gen-inductie en behandeling) is er voorafgaand aan het doden voor het verzamelen van weefsel kans op gering (en in een zeer beperkt aantal gevallen (<1%) matig) ongerief.
De uitvoering van het grootste aantal gedragstesten gaan (inclusief een eventuele voerbeperking of de toediening van tamoxifen of 'tool compounds') niet gepaard met meer dan licht ongerief. Bij een aantal testen (de 5.1 lid1c de Morris watermaze, 5.1 lid1c) is sprake van maximaal matig ongerief.
In een aparte groep dieren wordt door middel van audiogene prikkels de gevoeligheid voor het optreden van epileptische verschijnselen bepaald. Ook hier is sprake van matig ongerief (vooral bepaald door implantatie van de intracerebrale afleid electroden onder anesthesie).
Het optreden van spontane insulten wordt in beperkte mate verwacht en zal nooit resulteren in meer dan licht ongerief.
De commissie gaat er vanuit dat de keuze om de gedragsexperimenten uit te

voeren in de licht(niet actieve) periode ook een bron van additioneel ongerief is.

De commissie heeft uitgebreid met de indieners gecorrespondeerd en met de IvD vertegenwoordiger gesproken over de situatie of en wanneer er bij de voorgestelde experimenten zich situaties zouden kunnen voordoen waarbij door een stapeling van ongerief ten gevolge van verschillende interventies (elk op zich zelf resulterend in matig ongerief) uiteindelijk sprake zou kunnen zijn van cumulatief ernstig ongerief. De conclusie hieruit was dat dit in geen enkel geval te verwachten is, gezien de temporele scheiding tussen de handelingen.
Op basis hiervan is de commissie tot de conclusie gekomen dat het aangegeven ongerief (3916 dieren licht en 3850 matig) realistisch, voldoende onderbouwd en herleidbaar is ingeschat en geclassificeerd.

12. Het uitvoeren van dierproeven zal naast het ongerief vaak gepaard gaan met aantasting van de integriteit van het dier. Beschrijf op welke wijze er sprake is van aantasting van integriteit.*(Zie Praktische handreiking ETK: Stap 1.C2). (zie bijlage I voor voorbeeld).*
De integriteit wordt aangetast door het instrumentele gebruik als proefdier, de genetische modificatie, het geïnstrumenteerd worden, de huisvesting (beperking van fysiologische en ethologische behoeften) in een proefdierfaciliteit en in een beperkt aantal gevallen individuele huisvesting bijvoorbeeld in meetopstellingen en het doden van de dieren in het kader van het onderzoek.

13. Beoordeel of de criteria voor humane eindpunten goed zijn gedefinieerd en of goed is ingeschat welk percentage dieren naar verwachting een humaan eindpunt zal bereiken. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*.
De algemene en de experiment/interventie specifieke criteria voor humane eindpunten en de klinische parameters die daaraan ten grondslag liggen zijn benoemd en ingeschat. Als het gewicht van dieren bij de voerdeprivatie meer dan 20% bedraagt worden ze uit het experiment gehaald en weer op volledig voer gezet.
Er is bij de voorgestelde genetisch gemodificeerde lijnen altijd een risico op het optreden van epileptische insulten. Indien dit dreigt te resulteren in meer dan gering ongerief (bijvoorbeeld per dag meer dan 10 aanvallen korter dan 30 seconden of meer dan 2 aanvallen langer dan 2 minuten) is er sprake van een humaan eindpunt. Ernstig ongerief zal door te toepassing van de voorgestelde humane eindpunten in alle gevallen worden voorkomen.
De commissie kan zich vinden in deze inschatting en acht de humane eindpunten duidelijk en voldoende onderbouwd.

3V's

14. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte vervangingsalternatieven zijn. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*.

De aanvrager heeft voldoende aannemelijk gemaakt dat er voor de voorgestelde experimenten geen geschikte vervangingsalternatieven zijn. De complexe mechanismen die ten grondslag liggen aan de effecten van specifieke genen op hersenontwikkeling en de functionele impact hiervan kunnen alleen in vivo in intacte (complexe) organismen bestudeerd worden.

Er wordt binnen de instelling onderzoek gedaan naar de mogelijkheden voor toepassing van niet-operatieve terugplaatsing van embryo's in de fosters.

15. Beoordeel of het aantal te gebruiken dieren realistisch is ingeschat en of er een heldere strategie is om ervoor te zorgen dat tijdens het project met zo min mogelijk dieren wordt gewerkt waarmee een betrouwbaar resultaat kan worden verkregen. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*.

    Voordat besloten wordt tot gedragsexperimenten worden eerst alle moleculaire en elektrofysiologische experimenten uitgevoerd. Zoals ook al aangegeven in het antwoord bij C1 is het in het fundamenteel wetenschappelijk onderzoek niet mogelijk aan te geven hoeveel van elk van de aangegeven type experimenten uiteindelijk op welk moment en in welke vorm zullen worden uitgevoerd. De gedurende de looptijd van het project verkregen resultaten bepalen mede de keuze van de uitvoering en opzet van de vervolgexperimenten.
    De indieners hebben met betrekking tot het aantal dieren/experimenten op grond van hun ervaring en resultaten uit het verleden en (onderbouwde) verwachtingen met betrekking tot de toekomst hierover een inschatting gemaakt en hanteren vervolgens bij het bepalen van de proefopzetten de strategie ervoor te zorgen dat er met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog wetenschappelijk betrouwbare resultaten kunnen worden verkregen. Deze strategie is vooral gebaseerd op de ervaringen met het betreffende type experimenten in het verleden.

    De commissie heeft uitgebreid gesproken over dit aspect en heeft uiteindelijk geconcludeerd dat de wijze waarop de indieners hier nu vorm aan hebben gegeven enerzijds voldoende (realistische) handvatten biedt voor de commissie om een afweging te kunnen maken en tegelijkertijd voor de indieners nog voldoende vrijheid biedt bij de keuze en uitvoering van hun experimenten. Het aantal te gebruiken dieren is op basis hiervan ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet.
    De commissie acht binnen deze context de inschatting van het aantal te gebruiken dieren voldoende realistisch onderbouwd en navolgbaar.

16. Beoordeel of het project in overeenstemming is met de vereiste van verfijning van dierproeven en het project zodanig is opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C3)*.
    De indieners hebben duidelijk hun strategie met betrekking tot de toepassing van mogelijke verfijningen in hun onderzoek aangegeven. De commissie heeft dan ook geobserveerd dat bij het opstellen van de proefopzetten iedere keer weer op basis van eigen resultaten en die van hun collega's een duidelijke afweging zal worden gemaakt met betrekking tot de toepassing van mogelijke verfijningsalternatieven. De commissie onderschrijft deze strategie.

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

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Geef aan of dieren van beide geslachten in gelijke mate ingezet zullen worden. Indien alleen dieren van één geslacht gebruikt worden, beoordeel of de aanvrager dat in voldoende mate wetenschappelijk heeft onderbouwd. *(Zie Praktische handreiking ETK: Stap 1.C3; zie bijlage I voor voorbeeld).*
    In de wetenschappelijke geïnitieerde experimenten wordt gebruik gemaakt van zowel mannelijke als vrouwelijke dieren. In het (onwaarschijnlijke) geval dat er een duidelijk sekse gebonden verschil in fenotype wordt gevonden zullen experimenten

uitgevoerd worden in het geslacht met het sterkste fenotype. Deze situatie wordt niet verwacht.

19. Geef aan of dieren gedood worden in kader van het project (tijdens of na afloop van de dierproef). Indien dieren gedood worden, geef aan of en waarom dit noodzakelijk is voor het behalen van de doelstellingen van het project. Indien dieren gedood worden, geef aan of er een voor de diersoort passende dodingsmethode gebruikt wordt die vermeld staat in bijlage IV van richtlijn 2010/63/EU. Zo niet, beoordeel of dit in voldoende mate is onderbouwd. Licht uw beoordeling toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd *(Zie Praktische handreiking ETK: Stap 1.C3).*

    Alle dieren worden na afloop van de dierproef of in het kader van het project gedood. Dit is bij de wetenschappelijke experimenten noodzakelijk voor het verzamelen van weefsel (vooral hersenen) voor analyse.

    De dodingsmethode is conform bijlage IV van de Richtlijn 2010/63/EU.

20. Indien dieren worden gedood om niet-wetenschappelijke redenen, is herplaatsing of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is.

    Alle dieren in de wetenschappelijk geïnitieerde experimenten worden gedood om wetenschappelijke redenen (postmortem analyse van weefsel (vooral hersenen). Voor de studs en gevasectomeerde mannelijke dieren is sprake van continued use. De draagmoeders worden gedood in het kader van het project, hoewel niet om wetenschappelijke of welzijnsredenen. Dit is moreel problematisch, maar zeer moeilijk te vermijden omdat er geen wetenschappelijke of andere bestemming is voor deze dieren.

NTS

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

    De NTS is een evenwichtige weergave van het project.

D. Ethische afweging

1. Benoem de centrale morele vraag *(Zie Praktische handreiking ETK: Stap 3.A).*

    Rechtvaardigt het belang van het verkrijgen van wetenschappelijk inzicht in de rol van het UBE3A gen en het 15q11-13 gencluster bij de normale en afwijkende hersenontwikkeling waarbij ook aangrijpingspunten voor mogelijk therapeutische interventies zouden kunnen worden geïdentificeerd, het gebruik van 7766 (deels genetisch gemodificeerde) muizen en de hiermee gepaard gaande aantasting van hun integriteit en welzijn (licht ongerief (3916 muizen), matig ongerief (3850 muizen)?

2. Weeg voor de verschillende belanghebbenden, zoals beschreven onder C5, de sociale en morele waarden waaraan tegemoet gekomen wordt of die juist in het geding zijn, ten opzichte van elkaar af. Om dit proces te vergemakkelijken, kunt u de belangrijkste belanghebbenden en de belangrijkste waarden die in het geding zijn waarderen. U kunt dit verwoorden in termen van gering, matig of veel/ernstig voordeel of nadeel. Geef aan waarom de DEC bevordering van waarden (baten) voor de ene belanghebbende prevaleert boven de aantasting van waarden (kosten) voor de andere belanghebbende *(Zie Praktische handreiking ETK: Stap 3.B; zie bijlage I voor voorbeelden).*

Bij alle dieren (7766 muizen) is sprake van een aantasting van integriteit en welzijn. Bij maximaal 3850 muizen is er risico op matig ongerief en bij 3916 muizen is er sprake van licht ongerief. Het risico op het bereiken van een humaan eindpunt wordt zeer klein geschat.
Het voorgestelde onderzoek beoogt inzicht te verkrijgen in de rol van het UBE3A gen en het 15q11-13 gencluster tijdens de normale en verstoorde hersenontwikkeling en het identificeren van het neuronale substraat hiervan.
Naast dit onmiskenbaar fundamenteel wetenschappelijk belang is voor de groep patiënten met genetisch modificaties in het UBE3A gen (en hun naaste familie en de maatschappij) het belang groot.
Hoewel dat buiten de directe scope van dit project valt zou het beschikken over adequate behandelingen voor patiënten met afwijkingen in het UBE3A gen van groot persoonlijk en maatschappelijk belang zijn.
Voor de instelling 5.1 lid2h het uitvoeren van fundamenteel wetenschappelijk onderzoek in dit onderzoeksveld van groot belang. Niet in het minst voor de snelle vertaling van de wetenschappelijke resultaten naar klinische trials en uiteindelijk toepassing in de kliniek.

3. Beantwoord de centrale morele vraag. Maak voor het beantwoorden van deze vraag gebruik van bovenstaande afweging van morele waarden. Maak daarnaast gebruik van de volgende moreel relevante feiten: belang onderzoek (C4), kennis en kunde van betrokkenen (C7), haalbaarheid doelstellingen (C8), categorieën en herkomst dieren (C9), 3V's (C14-C18), ongerief (C10-13 en C19) en relevante wet en regelgeving (C2). Onderbouw hoe al deze elementen zijn meegewogen bij de beantwoording van de centrale morele vraag, zodanig dat het navolgbaar is zonder gedetailleerde kennis te hebben van het projectvoorstel *(Zie Praktische handreiking ETK: Stap 3.C; zie bijlage I voor voorbeeld)*.

De DEC is overtuigd dat de in dit project voorgestelde experimenten een bijdrage zullen leveren aan de kennis over de rol van het UBE3A gen op de hersenontwikkeling en het neuronale substraat dat hierbij betrokken is.
Naast dit onmiskenbaar grote fundamenteel wetenschappelijke belang zijn er uiteindelijk verschillende maatschappelijke belangen gediend met de resultaten van dit onderzoek. In eerste instantie het belang van de patiënten met afwijkingen in hun UBE3A gen. Het mogelijk reduceren van hun ziektelast dient naast een groot persoonlijke belang voor de betreffende patiënten ook een aanzienlijk breder maatschappelijk belang: de verminderde ziektelast bij de familie, de zorg en de maatschappij als geheel. Omdat voor UBE3A gen gerelateerde type ziektebeelden op dit moment nog geen therapie beschikbaar is, is uiteindelijk dit onderzoek ook voor de betrokken clinici van groot belang.
De DEC is van mening dat de directe belangen voor onderzoekers en de wetenschap en de mogelijke uiteindelijke belangen voor de betreffende patiënten, hun directe omgeving, de berokken clinici en de maatschappij, voldoende zwaar wegen om het schaden van de belangen van de proefdieren om gevrijwaard te blijven van een aantasting van hun integriteit en een licht of matige aantasting van hun welzijn te rechtvaardigen.
De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de directe doelstellingen binnen de looptijd van het project. De brede (klinische) inbedding van het onderzoek maakt de drempel naar eventueel verder klinisch onderzoek en uiteindelijk toepassing van de verkregen kennis in de kliniek zeer laag.
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 voorkomen zal

worden dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.
De DEC is van oordeel dat de hier boven geschetste belangen de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van aantasting van hun integriteit en in een deel van de dieren matig ongerief rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen de aantasting van integriteit en ongerief dat de dieren wordt berokkend, is in dat opzicht voldaan.

E. Advies

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

2. Het uitgebrachte advies is met een meerderheid van stemmen tot stand gekomen.
   Er is sprake van een minderheidsstandpunt wat zich primair richt op de belangen van de belanghebbende 'de wetenschap' (in termen van kwaliteit) en de daarmee verbonden belanghebbende 'de proefdieren' (in termen van aantallen en ongerief en aantasting van integriteit).
   Dit minderheidsstandpunt had in algemene zin niet betrekking op het belang van fundamenteel onderzoek naar het Angelman en Dup15q syndroom, maar was meer gericht op het open karakter van de aanvraag (de startmomenten van het onderzoek, de inherente onzekerheden met betrekking tot de keuzes voor bepaalde modellen en de vrijheid bij de keuzes in het kader van minimalisatie van de aantallen dieren en het ongerief bij het opzetten van de experimenten).
   Daarnaast was er de opvatting dat de belangen van de proefdieren geschaad worden door niet onderbouwd rekening te houden met de aangetoonde welzijnsconsequenties bij het uitvoeren van de gedragstesten tijdens de rustperiode.

   De meerderheid van de commissie heeft in haar afweging betrokken dat onzekerheid (zeker op een termijn van 5 jaar) inherent is aan de uitvoering van fundamenteel wetenschappelijk onderzoek en heeft bij het hierboven aangegeven 'open karakter van het project' ook zwaar de aantoonbare ervaring en kwaliteit van de wetenschappelijke infrastructuur binnen de onderzoeksgroep en de instelling en de kwaliteit van de in het verleden verkregen resultaten (bijvoorbeeld in de vorm van publicaties in high ranking tijdschriften en translaties naar de kliniek) meegewogen.

   Voor wat betreft de uitvoering van de gedragsexperimenten in de lichtperiode. Dit betreft een zeer algemene en breed toegepaste en geaccepteerde werkwijze. De commissie sluit niet uit dat deze werkwijze (dieren in een voor hen biologisch gezien 'vreemde' situatie plaatsen) onderdeel is van het model. In de correspondentie met de indieners, haar afweging en in haar advies heeft de commissie aangeven dat deze werkwijze resulteert in additioneel ongerief en (mogelijk ook) in een aantasting van de integriteit. (zie C11 en C12).

3. Omschrijf de knelpunten/dilemma's die naar voren zijn gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies zowel binnen als buiten de context van het project *(Zie Praktische handreiking ETK: Stap 4.B)*.
   Zie E2

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 neem telefonisch contact op. (0900-2800028).

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* 5.1 lid2h

☐ Nee > *U kunt geen aanvraag doen*

1.2 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 1.3

☐ Wijziging > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.1

☐ Melding > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.2

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

Naam instelling of organisatie	5.1 lid2h			
Titel, voorletters en achternaam van de portefeuillehouder	Titel	Voorletters	Achternaam	☐ Dhr. ☐ Mw
	5.1 lid2e			
E-mailadres contactpersoon	5.1 lid2e			
Titel, voorletters en achternaam van de diens gemachtigde (indien van toepassing)	Titel	Voorletters	Achternaam	☐ Dhr. ☐ Mw
E-mailadres gemachtigde				

Vul de gegevens van het postadres in.

Straat en huisnummer	5.1 lid2h
Postcode en plaats	
Postbus, postcode en plaats	

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	5.1 lid2e	☐ Dhr. ☐ Mw.
Functie		
Afdeling	5.1 lid2e	
Telefoonnummer	5.1 lid2e	

		E-mailadres	5.1 lid2e	
1.5	*(Indien van toepassing)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.	(Titel) Naam en voorletters	5.1 lid2e	☐ Dhr. ☐ Mw.
		Functie		
		Afdeling	5.1 lid2h	
		Telefoonnummer	5.1 lid2e	
		E-mailadres		
1.6	*(Indien van toepassing)* 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		
1.7	*(Optioneel)* Vul hier de gegevens in van de Instantie voor Dierenwelzijn	Telefoonnummer		
		E-mailadres		
1.8	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 3
		☐ Ja > Geef hier onder kort de wijziging en de onderbouwing daarvan weer. Geef in de originele formulieren (niet-technische samenvatting, projectvoorstel en bijlage dierproeven) duidelijk aan (bij voorbeeld in een andere kleur) waar de projectaanvraag wijzigt. Ga daarna verder met vraag 6.
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	1 - 11 - 2022
		Einddatum (t/m)	31 - 10 - 2027
3.2	Wat is de titel van het project?	Mechanisms underlying the pathophysiology of Angelman Syndrome	
3.3	Wat is de titel van de niet-technische samenvatting?	Mechanismen die ten grondslag liggen aan Angelman Syndroom	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) van voorkeur?	Naam DEC	5.1 lid2h
		Postadres	
		E-mailadres	

4 Factuurgegevens

4.1 (indien factuuradres afwijkt van de gegevens uit vraag 1.3) Vul de gegevens van het factuuradres in.

Naam:	Afdeling:	
Straat:		Huisnummer:
Postcode:	Plaats:	
Postbus:	Postcode:	Plaats:
E-mail:		

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

Ordernummer:

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel	Aantal bijlage(n) dierproeven 4
☒ 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 de Centrale Commissie Dierproeven (voor adresgegevens zie website)

Ondertekening door de portefeuillehouder namens de instellingsvergunninghouder of gemachtigde (zie 1.8). 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 C 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	5.1 lid2e
Functie	Gemandat
Plaats	5.1 lid2h
Datum	04 - 08
Handtekening	

5.1 lid2e

Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	Provide the title of the project.	Mechanisms underlying the pathophysiology of Angelman and Dup15q syndrome

2 Categories

2.1	Please tick each of the following boxes that applies to your project. Let op! De verplichte bijlagen verschillen per categorie. Op hetInvloket.nl leest u meer informatie over de verplichte bijlagen per	☒ Basic research ☐ Translational or applied research ☐ Regulatory use or routine production ☐ Research into environmental protection in the interest of human or animal ☐ 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 2.1.

The UBE3A gene plays an important role in a number of neurodevelopmental disorders, namely Angelman syndrome (AS; caused by loss of UBE3A)) and 15q11-q13 duplication syndrome (Dup15q syndrome; caused by duplication of a number of genes including UBE3A), as well as rare cases of autism spectrum disorders (ASD; caused by duplication of UBE3A or gain of function mutations of UBE3A).

AS is a severe neurodevelopmental disorder affecting approximately 1:20,000 births resulting in severe developmental delay (max. developmental age is ±2 years), intellectual disability, motor dysfunction, behavioural abnormalities including autism and impaired sleep cycle, and absence of speech. A large number of patients (80%) also suffer from epilepsy. AS is caused by mutations affecting the maternal copy of the UBE3A gene, which encodes a ubiquitin ligase that plays an important role in protein homeostasis in the cell. UBE3A fulfils its function by interacting with other proteins (targets) and attaching ubiquitin peptides to these targets, which can, amongst other things, mark the proteins for degradation.

To understand more about the basic biology underlying the pathophysiology of AS, we have carried out work under PLA AVD5.1 lid2h to identify the function of specific parts of the UBE3A protein, identify its target proteins and understand its role in neuronal function and brain development. We published work that showed the importance of correct localisation of the UBE3A protein to the nucleus, a property of UBE3A that is shared between mice and humans [1][2]. We found that the majority of UBE3A missense mutations as observed in patients rendered the nuclear UBE3A protein cytosolic which was enough to cause AS [3]. To study the specific role of each of the nuclear or cytoplasmic UBE3A protein isoforms, we have successfully generated a number of mouse lines in the previous 5 years that express only the nuclear or cytoplasmic UBE3A isoforms. In the coming 5 years we hope to use these mouse models to help us understand the role of each isoform. Being a ubiquitin ligase, one of the questions that still linger concerns the target proteins ubiquitinated by UBE3A. A major data set was created in a collaborative effect between our lab and the pharmaceutical industry, in which targets of UBE3A were found in a large scale spatiotemporal proteomic analysis [4]. This valuable data set is publicly available (https://www.angelman-proteome-project.org).

Despite these advancements, a lot is still unknown about the function of UBE3A in the different cellular compartments, and with which targets UBE3A interacts. We also do not know what makes UBE3A so essential to brain development. Additionally, the majority of AS patients carry a deletion that not only includes the entire UBE3A gene also but many other genes, present at the 15q11-13 locus. These patients present with a more severe phenotype making it important to disentangle the contribution of these additional genes within the AS pathophysiology.

Through imprinting, the UBE3A protein levels are tightly regulated. The importance of this tight regulation is apparent from rare patients that have multiple copies of UBE3A and suffer from autism and other neuropsychiatric disorders. Moreover, individuals carrying multiple copies of maternal 15q11.2-q13.1, the very same region that is deleted in most AS patients, suffer from Dup15q syndrome (1:5,000). Dup15q syndrome is in most cases a very debilitating neurodevelopmental condition, also characterized by intellectual disability, impaired motor coordination, autism spectrum disorder and epilepsy. The epilepsy in these patients can be severe and sometimes results in unexpected death (SUDEP). The penetrance and severity of the symptoms of Dup15q is driven by two factors: the number of duplications and whether the duplicated region is derived from the maternal chromosome or not. The symptoms are worst upon maternal inheritance of the duplications. The duplicated region includes *UBE3A*, the only gene within this region that is expressed solely from the maternal allele in mature neurons. Although this would implicate UBE3A as the main causative factor, patients with duplications of only *UBE3A*, as opposed to the entire 15q11.2-q13.1 area, do not show intellectual disability and epilepsy. Recently, we extended these clinical observations by showing that overexpressing UBE3A in mice also does not cause any major phenotypes, suggesting that there is a synergy between UBE3A and other gene products present in the duplicated region that underlies the Dup15q phenotypes.

There are currently no effective treatments available for AS and Dup15q and patients need life-long care. A better understanding of the fundamental biology and physiology underlying disorders involving UBE3A such as Angelman Syndrome, Dup15q and UBE3A-associated ASD can open up new directions for therapeutic strategies. These are important questions that we will address in the category 'Basic Research'. The category 'Basic research' encompasses all studies concerning the pathophysiology of the absence or overexpression of UBE3A and the role of neighbouring genes at the 15q11-13 locus as well as UBE3A target genes.

We have previously developed 5.1 lid2h [5][6], which makes this a very powerful tool for both drug testing as well as understanding the role of UBE3A in the brain. Work on mice carried out under PLA AVD5.1 lid2h has led to a publication in which we showed reversal of AS phenotypes treated with antisense oligonucleotides (ASOs) [7]. This was a proof-of-concept study that formed the foundation upon which the clinical trials are based that are currently performed on AS individuals at our center. Our lab holds AS mouse models that are already used for drug

testing on a regular basis. Importantly, our close connection to the clinic through 5.1 lid2h [redacted] enables us to translate preclinical findings of promising therapies to the clinic

3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The immediate goal of this project is to understand the role of UBE3A in brain development, with the ultimate goal to identify a treatment for Angelman syndrome and Dup15q syndrome. More specifically we have the following goals:

Aim 1: Investigate the cellular role of UBE3A in brain development.

The critical targets that are ubiquitinated by UBE3A ('UBE3A targets') are still poorly understood, and their identification remains a central part of our research. We have become very intrigued by our (unpublished) findings that UBE3A binds to the proteasome and could be a regulator of the proteasome. The proteasome is important for protein homeostasis as it rids the cell of aberrantly folded or excess proteins. Hence, if UBE3A is important for proteasome function, loss of UBE3A will likely affect many proteins. In this aim we will in particular study the genes/proteins that are regulated by UBE3A. Most of these experiments are performed in cultured cells, but some questions can only be addressed in neurons (ex vivo) or in living animals (behavior).

5.1 lid2h [redacted] Hence, 5.1 lid1c [redacted] Understanding the biological basis of UBE3A function in the neuron will be the first step in devising new therapeutic strategies.

Aim 2: Study the synergy between UBE3A and the neighbouring genes in the 15q11-13 gene cluster in both AS and Dup15q.

Although specific mutations only affecting the UBE3A gene result in AS, the majority (80%) of the patients harbour a large deletion affecting maternal chromosome 15, encompassing the 15q11-13 region (±6Mb) which includes UBE3A. The symptoms experienced by these patients are typically more severe compared to individuals carrying mutations only affecting the maternal copy of UBE3A, in particular with respect to epilepsy and their cognitive abilities. 5.1 lid1c [redacted]

The same region that is deleted in AS is present in multiple copies in Dup15q syndrome. As explained in 3.1, 5.1 lid2h [redacted] . 5.1 lid1c [redacted]

Aim 3: Identify brain regions that are affected by loss of UBE3A

Understanding which brain regions are affected by loss (or duplication) of UBE3A is important for two reasons: (a) Once we know which brain regions underlie the mouse behavioural deficits (for instance Ube3a mice have notable marble burying deficits), we can better translate these measurements to clinical trials. (b) There is a great interest in applying genetic treatments using viral vector approaches to

target specific brain areas. But which brain areas do we need to target with these vectors? And which brain areas contribute to the limited critical treatment period in which we can obtain a full behavioural rescue. In this aim, we will address these questions. These questions can only be addressed in animal models.

3.2.2 Provide a justification for the project's feasibility.

5.1 lid2h, 5.1 lid1c [redacted]

The feasibility of this project is further demonstrated by past performance. For AVD5.1 lid2h [redacted] we used approximately 5.1 lid2h [redacted] and 5.1 lid2h [redacted] (Appendix 1), 5.1 lid2h [redacted] (Appendix 3) which is now used in labs all over the world, and we have significantly contributed to our understanding of Angelman Syndrome (Appendix 2). 5.1 lid2h [redacted] is made available online as a searchable tool. 5.1 lid2h [redacted] (Appendices 2 and 3).

5.1 lid2h [redacted] Our average relative impact citation score is 6, meaning that our papers are 6 times more cited than the average paper in the field of neurodevelopment.

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

☒ No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

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

Social relevance: Angelman syndrome and Dup15q syndrome are very severe neurodevelopmental syndromes with an incidence of respectively 1:20,000 and 1:5,000 characterized by severe intellectual disability, intractable epilepsy, and behavioural problems, despite normal life expectancy. Hence the patients need life-long care. Little is known about the underlying pathophysiology, and currently there is no effective treatment. Even anti-epileptic drugs are often not effective in these patients, and the behavioural problems, absence of a regular day-night time sleep rhythm, as well as the inability to talk, puts a great burden on the families. Therefore, drugs that alleviate (some) of these problems are very welcome. For the identification of future therapies, we need to have a mechanistic understanding of the function of UBE3A in brain development.

'Scientific relevance:

Aim1: Although 88 genes involved in the Ubiquitin-Proteasome-System (UPS) are causally associated with neurodevelopmental disorders, we still have a poor understanding why mutations in these genes affect the brain so strongly while apparently not giving any problems in other organs of the body. It is likely that the UPS in the brain plays a very important part in the dynamic protein homeostasis required for synaptic plasticity. While there are over 600 E3 ligases, UBE3A (also known as E6-AP) is the prototypic HECT E3 type ligase (HECT stands 'Homologous to the E6-AP Carboxyl Terminus)' and is among the most studied E3 ligases. UBE3A is also a rather unique ligase since it binds tightly to the proteasome, a feature that is observed in only 3 out of the 600 E3 ligases. Also, the notion that UBE3A exerts its function in the nucleus, rather than the synapse, has really sparked the interest of the UPS and

neuroscience field. Almost nothing is known about the role and regulation of the UPS in the neuronal nucleus.

Aim 2: In addition to understanding the role of the UBE3A protein within the cell/brain region, a large gap in our knowledge concerns the role of the other genes that are also affected in large deletion/duplication patients. This includes the HERC2 gene, which encodes an E3 ligase that is known to interact with the UBE3A protein. Understanding such interactions, both direct and indirect, will provide us with important biological insight in UBE3A functioning, and ultimately how this impacts the severity of the AS and Dup15q condition

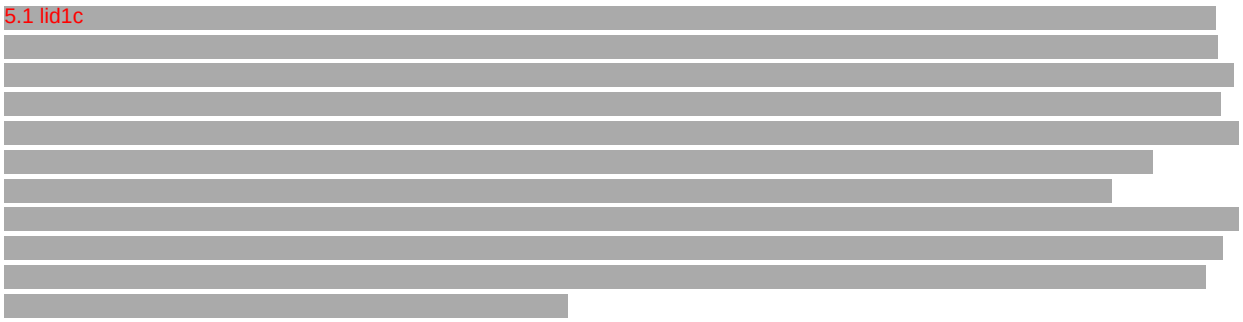
Aim3: Knowing which brain areas are affected by loss (or duplication) of UBE3a is not only of clinical interest but also of great interest from a behavioural neuroscience point of view. The strong phenotypes in our UBE3A mice along with the many tools to induce or reduce gene expression at great spatial and temporal resolution gives us a unique handle to probe which brain areas are involved in commonly used behavioural tests.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

Laboratory animals: The goal of this application is to request the use of animals to help us understand the biology behind AS and to develop drugs for patients. The animals obviously have a negative interest, as their integrity can be affected by genetic modification in addition to applying drugs and performing surgeries and tests, all potentially harmful to the animal. The animals are eventually killed as part of the experiment. The welfare of the mice will be at most moderately affected during our experiments and it is our moral duty to ensure that the discomfort the animals endure is kept to a minimum.

Patients and parents: Patients (and indirectly their family members) with AS/Dup15q will hopefully profit from our experiments. Despite this clear positive interest, they may also need to provide cells (negative interest), often in the form of a vial of blood, from which iPSC derived cells will be generated. In addition, there are no guarantees that we succeed in identifying a treatment, and even if we do so they carry a significant risk when these therapies are applied to the patients. This risk is reduced but not excluded upon in vivo testing in animals.

The lab: The lab has a scientific interest in deciphering the aetiology of AS and Dup15q Syndrome. The results obtained from our research are not only beneficial to patients, but also to the scientific community at large as it helps us to understand UBE3A in addition to its interacting proteins/factors and the other proteins encoded by the neighbouring genes. The lab also has a strong scientific interest in understanding the role of the proteasome in the neuronal nucleus (which is an entirely unexplored topic) and understanding which brain areas contribute to commonly observed mouse phenotypes. The lab generates its income from its publications, as they will facilitate funding of new projects. With respect to current lab funding sources, 40% comes from government funding, 40% from patient organisations and 20% from industry.



Society: In terms of the societal impact, AS is a debilitating disorder that has a great impact on the afflicted individuals but certainly also on the care-givers. A treatment for some of these disorders may have a positive impact on the patient and thus on our society at large, but could also influence health costs.

Industry: some of the drugs will be developed together with the pharmaceutical industry. They will financially profit from our studies but it will increase the chance of developing a drug and get it approved for clinical trials.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

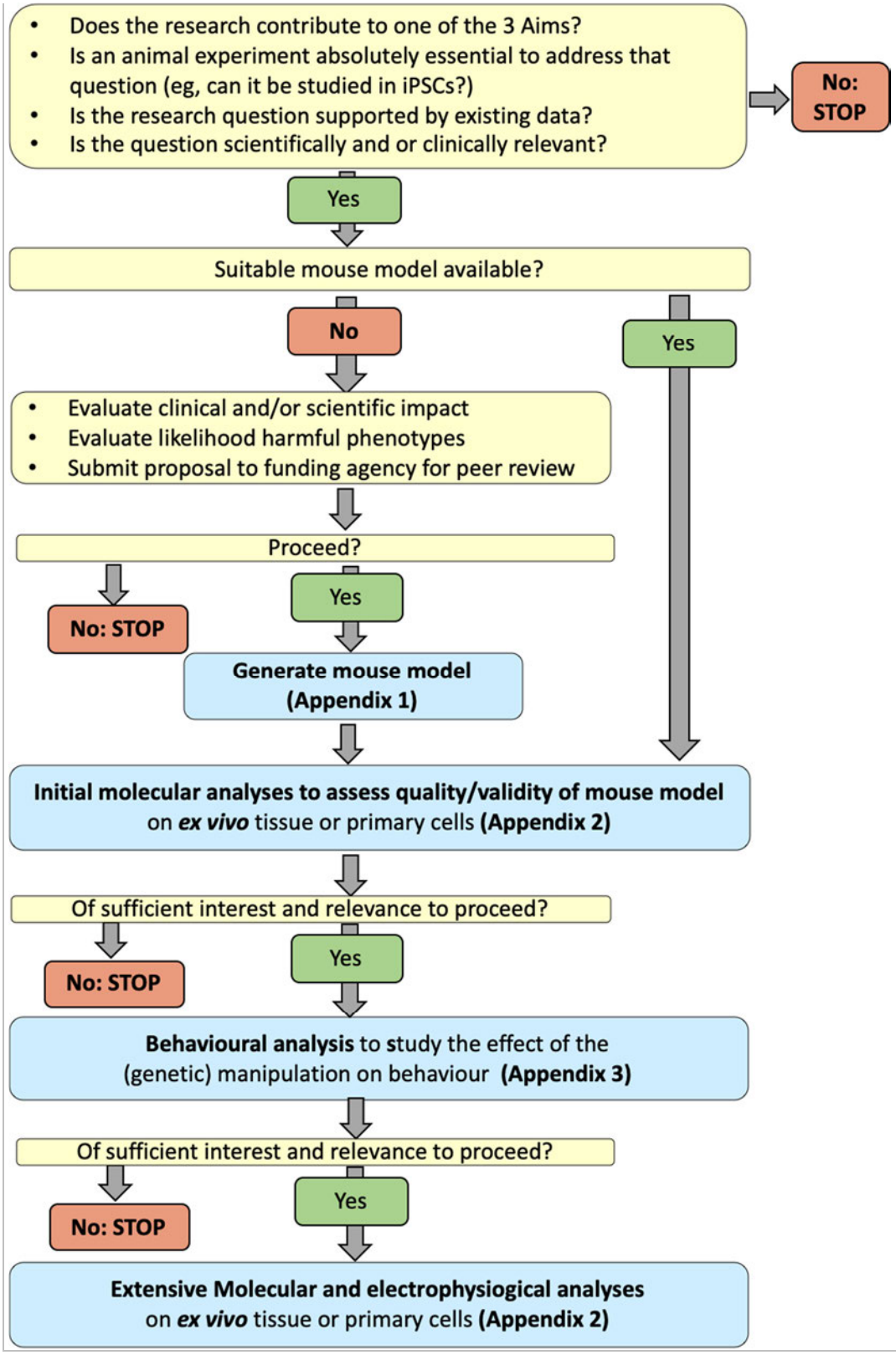


Figure 1: Overall strategy

Our research focuses on the pathophysiology (and function) of UBE3A and its synergy with genes in the 15q11-13 gene cluster , and its role in the various brain regions (see 3.2.1 Aims 1-3). Where possible our lab primarily utilises molecular and cellular studies **that do not require animals to address these questions**. Since the UBE3A-related disorders are all disorders of the brain, we focus our work specifically on neurons (including inducible pluripotent stem cells (iPSCs)). However, since UBE3A is critical for early postnatal brain development, the loss/overexpression of UBE3A and other genes in the 15q11-13 gene cluster cannot be faithfully modelled in a dish. Hence, we still need animals for certain research questions.

For this PLA we will primarily focus on characterizing the following (already available) mouse lines:

With the exception of the first line, 5.1 lid1c or Transgenic Cre driver lines) to address scientifically relevant questions. These crossings would yield far more possibilities than we can (and want to) investigate in 5 years. Also, the number of experiments that we can do is strongly dependent on the nature of the experiment. Hence, we will have to make choices based on the scientific relevance, data that we acquired, and on data that others published during this PLA. Finally, funding agencies will ultimately have a critical voice in what we actually can do. Together, these factors make it difficult to compile a complete list of experiments to be carried out in the next 5 years. The numbers listed in the appendixes are therefore based on a realistic prediction of what we can and will do in the next 5 years, based on previous experiences. In terms of discomfort, there is no a priori difference in which strain we are characterizing.

To address our research questions we will use the lines above to perform the following mouse experiments:

A. Generation of mouse models (Optional; Appendix 1): In order to study the mechanisms underlying AS and DUP15q it is critical that these mice have high construct validity. Hence, the genes that are targeted in our mice should be very similar to the mutations as observed in the patients. In addition, to justify studying these animals as models of disease, the mice also need to show high face validity, meaning that the phenotypes must resemble phenotypes observed in patients. When we want to generate mice to test a specific scientific question (i.e. does gene X in the 15Q11-13 locus contribute to the disease), we should thoroughly investigate (by extensive literature studies and in vitro experiments) if that gene is indeed of high scientific interest and potentially of critical relevance to brain function to justify a new mouse model that specifically addresses that hypothesis

B. Molecular and electrophysiological analyses on tissues/primary cells (Appendix 2): Although we can now generate iPSC-derived neurons, these neurons do not show the same mature properties of neurons as can be obtained from mouse primary brain tissue. Also, electrophysiological measurements require fine-tuned synaptic connections that are not present in neurons grown in a dish, hence we need brain slices to measure the deficits of these neurons. These experiments will help us assess the role of UBE3A at the molecular and cellular level and help us address the questions for Aims 1-4. The primary

neuronal/astrocytic cultures will be used to test targets or interactors of UBE3A in knock-down or overexpression studies.

C. Mouse behavioural studies (Appendix 3): Only when molecular studies look promising and warrant further research to investigate how the manipulation affects behaviour (which is the final output of the brain), we will perform behavioural assays. We will most often use our well-established behavioural test battery [6], which is now used by many labs all over the world to assess behavioural deficits caused by loss of UBE3A (**Aim 3**). In case we have identified a putative UBE3A target (**Aim 1**) we may perform a proof of principle study, by crossing AS mice with a mutant of the identified target (reduced- or overexpression) to test the effect of such a double mutant. Behavioural testing is also needed to assess the effect of neighbouring genes in the 15q11-13 gene cluster (**Aim 2**) and to identify which brain regions are underlying the behavioural deficits caused by loss of UBE3A (**Aim 3**). Some of these experiments may require the injection of ASOs or AAV viruses in the brain to manipulate the expression of certain genes in our mutants (eg. putative UBE3A targets or specific genes in the 15q11-13 region).

3.4.2 Provide a justification for the strategy described above.

Appendix 1: Generation of mouse models: During the next 5 years, new information may become available from our own data, partners, meetings or the scientific literature. Based on the new knowledge, we may deem it necessary to generate or import a new mouse line to better model the disorder or allow us to get a better understanding of the function of proteins involved in AS or Dup15q.

Appendix 2: Molecular and electrophysiological analyses on tissues/primary cells: With the aim to understand the pathophysiology of AS, we would like to interfere with the expression of not only UBE3A but also of its interactors /targets. To achieve this, we plan to:

1. use "tool compounds" such as antisense oligos (ASOs), short hairpin RNAs (shRNAs), short interfering RNAs (siRNAs), short activating RNAs (saRNAs), microRNAs (miRNAs), DNAs or viruses to downregulate or overexpress the genes in question. Which of the aforementioned tool compounds will be used depends on the level of downregulation/overexpression we need. Since AS is a neurodevelopmental disorder, we would most likely carry out our intervention with tool compounds at an early age, such as in utero or directly after birth and administered using intracerebroventricular injections.
2. Setup breedings between our current mouse lines, or to achieve difference is gene dosages. As an example, the effect of levels of (neighbouring gene) interacting proteins such as HERC2 relative to UBE3A can be looked into by crossing our dup15q mouse line mice with our UBE3A overexpressing lines.

Appendix 3: Mouse behavioural studies: These experiments allow us to determine the face validity of our animal models, ie. do they display a phenotype and whether the phenotype(s) are stronger or milder. Also here, the administration of tool compounds may help us determine the role of UBE3A or (neighbouring gene) interactors.

In terms of animal wellbeing**, the strategy is designed in such a way as to:**

(a) keep the animal numbers as low as possible. When possible, we use **genetically engineered (immortal) cell lines or iPS cells for initial charachterization or for testing of tool compounds.** Another example to keep the animal numbers low is the isolation **and storage of tissue** after each experiment (when possible), and **to perform brief molecular studies to assess the validity of the line before deciding to do behavioural studies. And only when we observe behavioural deficits will we perform detailed molecular and electrophysiological studies.**

(b) Keep discomfort scores as low as possible. The initial work is first performed in vitro. This reduces the number of in vivo experiments. We will **first perform a limited molecular**

characterisation to study the effect of our (genetic) manipulation, to keep discomfort as low as possible. If needed, we then proceed to electrophysiological studies. Only when we have found molecular or electrophysiological changes that warrant behavioural studies to study the effect on brain function, we will **finally perform behavioural studies**

(c) Avoid the generation of new animal models when possible. We will only generate mouse models if they are not available elsewhere and if they address a major question in the field that is of large clinical or scientific value. As the generation and characterization of new models is very expensive, the final decision is ultimately decided by the reviewers of our grants. All our mouse models are made available to the field before or upon publication.

3.4.3 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	Generation and breeding of (new) GA mouse lines with possible discomfort
2	Collection of mouse tissue
3	Behavioural analysis of mice
4	
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 Type of animal procedure: Generation and breeding of (new) GA mouse lines with possible discomfort

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.

To understand the fundamental mechanisms underlying the pathophysiology of UBE3A related disorders, we make use of mice to perform ex-vivo and in vivo experiments. Preferentially, these studies will be carried out on mouse models that already exist. When, after a literature review and also consultations with our extensive international network of partners, a suitable mouse line is not found, we will review the need to make a mouse model ourselves. Important factors in such an evaluation are the potential scientific and/or clinical impact of the mouse model, whether or not we expect a harmful phenotype. We will then submit a grant proposal that is subjected to peer review (typically patient organization, ZonMw, EU). If awarded, we will proceed to design and generate a new genetically modified (GA) line.

De novo generation of new GA lines: The genetic modification is applied to embryos or embryonic stem cells, and GA animals are created by:1) injection of GA embryonic stem cells (ES) in blastocysts. 2) Injection of DNA/RNA constructs in oocytes. 3) Modification of oocytes by new techniques for gene editing, such as those employing CRISPR/Cas9. We anticipate to **generate a maximum of 2 new lines** in the next 5 years.

Breeding of a GA line with a mild harmful phenotype: Currently, none of our lines has a harmful phenotype. However, it is possible that we will generate or import GA mice with a mild harmful phenotype. We anticipate to **breed maximally 1 line with a mild harmful phenotype** in the next 5 years.

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

Table 1 Types of procedures and level of discomfort*)

	Gender	Goal	Procedures	Discomfort
1. Donor females	F	Embryo isolation	Mate with fertile stud / Plug check / Cull mouse by cervical dislocation / Harvest embryos, i.e. zygotes, morulae or blastocysts	Mild
		Oocytes isolation	Superovulation (2 i.p. injections, SOP) / Cull mouse by cervical dislocation / Harvest oocytes	Moderate
2. Fertile stud male	M	Fertilization	Mate with female donor mouse (max. 3x per week, 4 months active)	None
3. Vasectomized stud male*	M	Mock fertilization	Mate with foster mother (continued use)	Moderate (continued use)
4. Foster mother	F	Oocyte or embryo implantation	Mate with infertile stud male / Plug check next day / Surgical embryo implantation under injection anesthesia and pain relief (SOP) / Birth of GM pups / Wean litter / Cull mouse	Moderate
5. Founders	M & F	Identification DNA extraction and analysis	Distal phalanx clipping of mouse at 4-7 days after birth (SOP)) for identifying and genoptyping. In exceptional cases, an additional clipping of the ears, tail tip clipping or a blood sample is needed for analysis of the genetic alteration.	≤moderate<1%
6. Harmful phenotype	M & F	Breeding /maintaining	Animals are maintained /bred with a mild harmful phenotype	Mild

*) Vasectomized stud males will be used from the cryopreservation/rederivation program running in the facility

Four procedures involve treatment of the mice: 1) superovulation, 2) implantation 3) vasectomy and 4) distal phalanx clipping or clipping of the ear.

Superovulation (oocytes): Approximately 5-week-old female mice receive 2 i.p hormone treatments, 48h apart. Based on our experience, superovulation at this age results in a maximum number of fertilised oocytes of good quality for most laboratory mouse strains. Superovulation of older mice dramatically reduces the number of oocytes. After the last treatment, the female mouse is mated with a stud male. Next day a plug check is performed. Plugged females will be collected and set aside till usage. Plugged female mice are culled 0.5-3.5 days post mating and oocytes are isolated. SOPs apply.

At the 5.1 lid2h [redacted], the choice to not super-ovulate donors prior to blastocyst isolation is a conscious one and is based on the fact that in their experience the quality of blastocysts is much lower after superovulation when compared to natural mating. In the end in turns out that a lower number of blastocysts but of higher quality vs higher number of low-quality blastocysts equates to an equal number of mothers used with the added benefit that the discomfort is lower (mild vs moderate). Also, important to note is that with natural matings, only plugged mothers are used.

Implantation: Two types of implantations are performed. 1) oocyte/2-cell implantation in the oviduct of a foster mother. 2) blastocyst implantation in the uterus of a foster mother. Surgery is performed on 8-16 week old plugged female mouse (weight 18-30g) following SOP and takes on average 20 minutes per mouse. In brief, a 1 cm incision is made parallel to the dorsal midline to expose the oviduct and uterus.

For the oocyte/2 cell implantation: The infundibulum is located and using a fine glass capillary pipet 20-25 oocytes/2-cell embryos are inserted into the oviduct.

For the blastocyst implantation: With a syringe a small hole is made in the uterus wall. With a small glass capillary pipet 8-10 blastocysts are inserted through the hole into the uterus.

Next the oviduct-uterus is placed back in the abdomen and the peritoneum is closed with 1-2 sutures. Wound clamps are used to close the skin, which are removed 8- 10 days after the operation. Mice are anesthetised and post operation pain relief is administered.

We are aware of the non-surgical embryo transfer (NSET) procedure which potentially lowers the discomfort of blastocyst recipients. The procedure has been tested in our facility but results in a lower number of pups compared to surgically placing back the blastocysts. So, although the present procedure of surgical implantation of blastocysts does lead to an increased number of donors and foster mothers, ultimately it does reduce the number of repeated injection attempts.

Distal phalanx clipping: Distal phalanx clipping of mouse at 4-7 days after birth following SOPs for identifying and genotyping

Selection of the most suitable founder lines: Only those newly generated founder lines that have germline transmission AND an appropriate level of GA effect (normally expression of the GA gene in specific tissues/cell types) will be selected to produce offspring for procedures in the other appendixes. Assessment of an appropriate level of GA effect may include (trans)gene (in)activation by eg tamoxifen treatment, continuously or intermittently and via different routes (eg. in max 4 months in feed and/or water; max 5 x IP (5ul/g), or exceptionally - when other methods do not produce the desired result - once ICV (max. 5ul) and subsequent target tissue analyses.

Initial Welfare assessment: As described in the EU directive (2010/63/EU: corrigendum 24-01-2013), various parameters are checked daily. New mouse lines will be monitored for two generations to determine whether there is a harmful phenotype. In those mouse lines that have a harmful phenotype, the breeding of affected animals itself will be registered as (a part of) a procedure. Mouse lines with a more than mild harmful phenotype will not be included in this project.

Maintaining a colony of a GA line with mild harmful phenotype (maximum mild severity): We request one group of mice to allow breeding and maintaining one line with a mild harmful phenotype.

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

Statistical analyses are typically not being performed for these kinds of experiments, except for testing whether alleles transmit in a Mendelian fashion.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice GA	N/A (Annex I species specifically bred)	Pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129
	mice normal	N/A (Annex I species specifically bred)	adults		Both	No	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	Mus musculus: The body of knowledge on the mouse brain is large, and the development of the central nervous system exhibits a high degree of similarity to that of the human CNS, which makes the mouse a good model. Another important factor is the technology to make genetic changes, which allows us to use GA mice as models for UBE3A related disorders.
Origin	All animals are bred in the institute or come from a registered supplier and are housed under the same standard conditions. Animals will be socially housed whenever possible

Life stages	Mus musculus: genetically altered (GA) and non-GA animals of defined standard genetic backgrounds, both male and females, typically up to an age of 240 days. Both females and males must be sexually mature to be used in these experiments for them to act as donors (embryo/oocyte/sperm) or as foster mothers. Adult founders will be used for breeding the F1.
Number	Mus musculus: normal (non-GA) animals as well as GA mice of defined standard genetic backgrounds, both male and females, typically up to an age of 240 days. All animals are bred in house or come from a licensed commercial supplier. We estimate to generate a maximum of 5.1 lid1c [redacted] 5.1 lid1c [redacted]) primarily involves selection on the basis of DNA sequence. After selection and breeding of the correct founder lines, further characterisation at both the genomic level as well as the protein level is necessary. In our experience with generating GA mice, more extensive characterisation of the inducible allele in the offspring from of each founder is necessary and how this affects protein levels needs to explored. This involves crosses with Cre- expressing lines to obtain tissues to test on Western blot, immunofluorescence labelling etc. 5.1 lid1c [redacted] **Maintaining a colony of a GA line with mild harmful phenotype:** All our current muse models, including both the large 3.5Mbp deletion AS model as well as the large duplication (Dup15q) mouse model **do not** show any harmful phenotype and therefore we anticipate that the newly generated lines or imported lines are likely to have no harmful phenotype as well. We can, of course, not guarantee this to be the case for the new lines to be generated or imported. 5.1 lid1c [redacted]
Gender	For the majority of experiments, mice of both genders can be used. There are exceptions such as egg donors and fosters being female and vasectomised males being male for obvious reasons.
Genetic alterations	For the generation of mouse models, it is important that the mutation encountered in patients is also introduced into the mouse genome to create a model with a high construct validity. This in turn increases its face validity. It is not possible to mention which mutations will be modelled here but will be identified in the study-plans.
Strain	We will use normal (non-GA), genetically altered (GA) and wild-type control mice in different standard genetic backgrounds (eg Bl6, 129 and FVB) or combinations (eg F1 Bl6 and 129) thereof depending on how the genetic background affects the phenotypes we see in the strains. The strains to be used will be mentioned in future study-plans.

C. Accommodation and care
Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?
☒ Yes
☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

Surgical procedures, including embryo transfer and vasectomy, will be carried out under general anaesthesia with adequate peri and postoperative analgesia.

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

In many cases it is difficult to predict exactly what the effect will be of an alteration of the DNA sequence of the mouse genome. From our experience, none of our AS mouse models (6 models thus far) show any deleterious effect on their well-being, and phenotypic changes we measure are only observed through advanced experimental testing where specific phenotypic modifications can be detected. However, since we may also generate models involving genes other than Ube3a, in 40% of the newly generated GA lines we can potentially expect a mild harmful phenotype such as susceptibility to epilepsy. It is difficult to predict beforehand the severity of the phenotype we expect as this can differ between humans and mice. When mouse lines do experience an intrinsic discomfort severity beyond mild, the line will no longer be used and the generation of an inducible line will be considered.

Explain why these effects may emerge.

These effects may emerge because of the role of the gene in brain development and their resemblance with AS/ Dup15q patient (of which most have epilepsy).

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

If unexpected adverse effects are observed, immediate action will be taken. This could be through removal of a specific animal from the experiment, or even termination of the entire GA mouse line in favour of a more refined model (e.g. inducible mutant).

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

All mice will be observed daily with respect to several parameters (overall appearance, size, confirmation and growth, coat condition, behaviour, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013.

Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:

1. Stunted growth (>15% reduced weight compared to littermates).
2. Moderate circulatory or respiratory problems.
3. The development of a clinical neurological disease that results in sustained suffering **(**eg they display deviant behaviour such as abnormal aggression, ataxia, lethargy or epilepsy**)**.

For animals that underwent surgery, we will also humanely kill the animal if two days after the start of the experiment the animal has a decrease in body weight of maximally 20%, with a recovery within 2 days post-surgery to a max of 10% (relative to their weight at the start of the experiment).

Indicate the likely incidence.

Less than 1%: We have extensive experience with generating new GA lines in the last 20 years, which include all our current Ube3a related models including both the large deletion AS model as well as the large duplication (Dup15q) mouse model. So far, none of these lines show any harmful phenotype and very rarely do we have to kill an animal because the above criteria are met. Since breeding of animals with abnormal behaviour/appearance increases the likelihood of affected offspring, animals are typically killed long before the humane endpoint criteria are met.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Donors: multiple injection of hormones to induce super-ovulation. Humanely sacrificed and eggs harvested (severity: Mild)

Fosters: These mice will undergo abdominal surgery to place back the blastocysts or injected eggs. Humanely sacrificed after weaning (Moderate) Vasectomised males: Males undergo abdominal surgery for sterilisation purposes (Moderate)

Offspring and breeding: These mice do not undergo any treatment. There is a potential for breeding with a mild harmful phenotype but that is difficult to predict (Severity: mild). The emergence of a mild harmful phenotype depends on the protein involved, and can cause for example, mothers to be more prone to stress and becoming more aggressive. As a consequence, some of the pups may be cannibalised by the mother within the first 14 days after birth. Mutations introduced into some mouse lines may result in an increased seizure susceptibility the frequency of which is at most 5/day This is determined during the extensive analysis of each line when mice will be observed with cameras and footage analysed to detect the presence of seizures. When the severity of the experienced harmful phenotype is deemed too high, the mouse line will no longer be used and the generation of an inducible mouse model may be considered (Severity: Mild).

All mice are humanely killed.

Mice normal

Severity	#	%	Treatment Groups
3 Moderate	160	80%	Female donors
3 Moderate	30	15%	Foster moms
2 Mild	10	5%	Vasectomized stud males (will be used from cryopreservation/rederivation program, hence not included in total)

Mice GA

Severity	#	%	Treatment Groups
2 Mild	100	14%	Founders
2 Mild	100	14%	tissue collection for analysis F1
2 Mild	500	71%	Maintenance of GA line with a mild phenotype

G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to modify the gene and to study the effect of the genetic modification on brain development and brain function. To date, studying GA mice is the only reliable method to investigate the function of a gene in brain development and brain function *in vivo*. Before we embark on choosing the genes

	for generating novel GA lines, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation.
Reduction	The vasectomised males used in this study also participate in the cryopreservation/rederivation program that is ongoing in the transgenic core facility, thereby obviating the need to use a separate cohort of mice.
Refinement	The lab has obtained extensive experience in the last 2 decades in the type of procedures been described and knows how to determine when mice are approaching humane end points such as weight loss, trembling, fur condition etc. The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of neurodevelopmental disorders, and participate in scientific meetings. Also, we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study. We continuously strive to reduce the discomfort the mice are subjected to, and although some labs do use the NSET procedure, a non-surgical procedure to place blastocysts back into the recipient, experience with this procedure in our facility resulted in a lower number of pups compared to when we used the surgical procedure, described in this application. We do recognise that the present procedure of surgical implantation of blastocysts does involve a higher discomfort to the mice, we believe that the higher number of mice/injection obtained in our hands does ultimately reduce the number of repeated injection attempts.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

A number of the animals will be humanely killed after general anesthesia, eg. egg donors and foster mothers (at weaning) in the course of the experiment. Non-GA offspring will be killed before weaning.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☒ No > Describe the method of killing.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia. This causes least pain andstress for the animals

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons, as part of the procedures or to resolve welfare concerns.

Centrale Commissie Dierproeven

Appendix
Description animal procedures

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- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 2 — Type of animal procedure: Collection of mouse tissue

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.

To address the research questions of this application, we will make use of *ex-vivo* experiments as much as possible. This appendix describes the collection of

1) Embryonic brain tissue to setup primary neuronal cultures will be used to determine the role of UBE3A protein in the different cellular compartments (**aim 1**) and to determine the effect of AS mutations or the role of neighbouring genes (**aim 2**) on selected readouts.

2) These same questions will also be investigated at different ages in vivo, by collecting brain tissue and subjecting these to various biochemical and electrophysiological analyses (**aims 1-2**). In addition, brain tissue is also required for molecular or electrophysiological studies to study the role of UBE3A in specific brain regions (**aim 3**) To address these aims, we will use normal and GA (single, double or triple) mice. In order to obtain the tissues, animals are humanely killed after general anaesthesia, and tissues are dissected for further analyses using biochemical/molecular, electrophysiological and imaging methods (microscopy analyses).

For **all aims,** we may administer Tamoxifen to induce expression of Floxed alleles. We may also administer tool compounds through 5.1 lid1c

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

Tissue collection of >P21 mice. Mice will be humanely killed after general anaesthesia.

Tissue collection of newborn animals: In case we need to collect tissue from newborn mice for subsequent analysis, pups are rapidly cooled on ice water to provide hypothermia-anaesthesia, and decapitated to obtain the brain for further analyses.

Administration of tool compounds to modulate gene expressions: Prior to tissue collection, animals may be subjected to **maximally 5 IP injections of tamoxifen** (maximum volume of 5ul/g) to induce brain- specific gene deletion/activation in the case of floxed alleles.

Animals may also be subjected to the administration of the molecules to modulate specific gene expression levels. 5.1 lid1c

Administration via ICV in neonates: this involves subjecting the neonates to hypothermia-anaesthesia using iced water and injecting the compound directly into the lateral ventricles. The whole procedure takes about 5 minutes after which the pups are placed under a heating lamp to recuperate.

In older mice, administration of tool compounds involves placing the mouse under general anesthesia with analgesia, moving the skin to allow the drilling of a small hole in the skull using the stereotactic device followed by suturing of the skin and wound care. The whole procedure takes approximately 30-40 min.

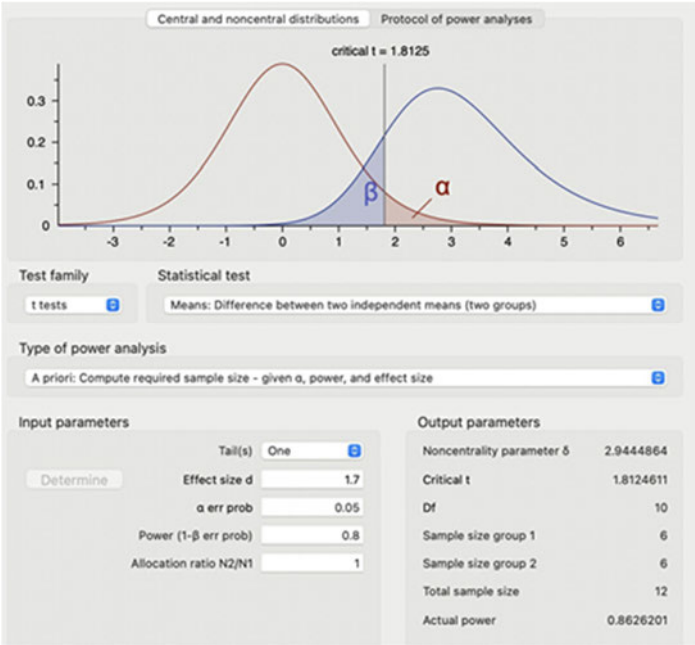
It is important to note that the cumulative discomfort endured by each animal due to the administration of these compounds will never exceed moderate discomfort.

For imaging analysis, some of the above mice will be sacrificed and subjected to transcardial perfusion after general anaesthesia (using an overdose of Nembutal (Pentobarbital)).

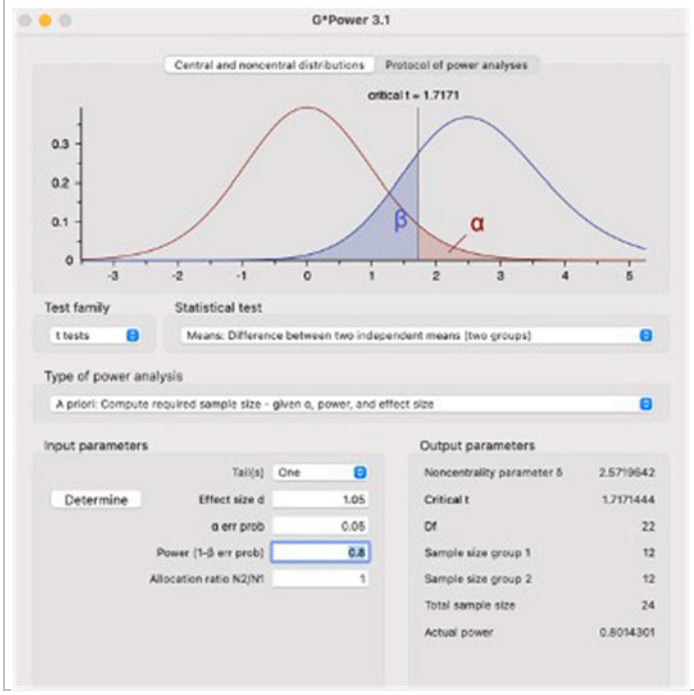
Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We use tissue for a large variety of experiments, each requiring different analyses and sample sizes. In most cases the number of animals will be based on literature and/or (more likely) on our previous experience with similar experiments. The experiments are often of explorative nature and will be carried out using a minimum number of mice per group to detect meaningful effect sizes (β of .8 and an alpha (α) of 0.05). The numbers are mostly based on previous experience and strongly depend on the outcome measure. The experimental design of each experiment including the numbers of animals and type of analysis will be submitted to the IvD.
Through careful randomization we try to minimize the number of animals by reducing the effects of potential differences in age, sex and litters. For our statistical analysis we do not take such possible differences into account, which, for this type of explorative experiments is the standard in the field.

As an example, for molecular Western blot analyses in which we want to be able to detect a difference of 20% of the means, we typically need 6 mice/group:



As an example for electrophysiological analyses in which we want to be able to detect a difference of 20% of the means, we typically need 12 animals/group:



B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice GA	N/A (Annex I species specifically bred)	Pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129
	mice normal	N/A (Annex I species specifically	Pups and adults		Both	No	eg. Bl6, FvB and 129

		bred)					

Provide justifications for these choices

Species	*Mus musculus*: Knowledge about the mouse brain is large, and the development of the central nervous system exhibits a high degree of similarity to that of the human CNS, which makes the mouse a good model. Another important factor is the technology to make genetic changes, which allows us to use GA mice as models for neurodevelopmental disorders.
Origin	All animals are bred in the institute or come from a registered supplier and are socially housed, whenever possible, under the same standard conditions. This ensures the animals are genetically pure and free of known infectious agents.
Life stages	Mice at all stages (from E14.5, neonates, juvenile, adolescent or adult) will be subject to biochemical, immunohistochemical, ePhys analyses after treatment with therapeutic/vehicle. This allows us to properly study the different developmental stages of the mice. The chosen age will depend on the functional test and whether the age is suitable for the test. The treatments described for the induction of the GA alleles and/or treatments with drugs will in some cases begin before parturition, to study the effects on the embryos during development.
Number	The numbers detailed below are a maximum we will expect to use. The actual numbers and conditions will be outlined and justified in the study-plans presented to the IvD for consent prior to performing any experiment. In the proposed experiments we will make use of a maximum of 6 GA mouse lines. Below we delineate the number of mice needed for each type of experiment. Typical group sizes: To address a research question using neuronal cultures, we typically use 5 independent cultures (5 dams, with on average 4 foetuses each (6 for normal, 4 for GA animals). To perform a detailed biochemical (e.g. proteomics, Western blots, kinase assays), genetic (e.g. gene expression (RNA-seq) analysis) and imaging analysis (e.g. immunofluorescence) we typically need 6 animals per group. For slice electrophysiology we typically record a number of different electrophysiological parameters from different cell-types, different brain areas, and different developmental ages. From previous experience we know that the recording of a single measure typically requires 12 animals per group. **I. Animals for neuronal cultures** 5.1 lid1c In Aims 1 and 2 we look into how specific mutations lead to a change in localisation and how UBE3A interacts with its "targets" in addition to deciphering the role of UBE3A in the nucleus/cytosol/synaptic compartments. We estimate that over 5 years we will perform 20 studies involving GA animals and 20 studies involving normal animals. With a group size of 5 dams per study, this requires: 5.1 lid1c **II. Animals for tissue collection without additional procedures or discomfort** 5.1 lid1c To charachterise our recently developed mouse models and future mouse models we will perform electophysiological analysis and molecular analysis on mice with different genotypes. Depending on the used genotypes, most experiments have 3 groups (WT, HET, HOM), 4 groups (WT, Het; plus or minus Cre) or up to 8 subgroups when double mutants are used. **For practical reasons we use for all our calculations 4 groups.** 5.1 lid1c We expect that in the coming 5 years we will submit 40 study plans to charachterize mice

	using molecular biology. Hence, 5.1 lid1c **III. Animals for tissue collection with a mild harmful phenotype** 5.1 lid1c Although currently none of our animals has a harmful phenotype, it is possible that interbreedings of current mutants or generation/import of novel GA lines yield animals with a mild harmful phenotype. We expect that in the coming 5 years we will submit 2 study plans to charachterize mice with a mild harmful phenotype using electrophysiology. 5.1 lid1c We expect that in the coming 5 years we will submit 5 study plans to charachterize mice with a mild harmful phenotype using molecular biology. Hence, 5.1 lid1c **IV. Animals for tissue collection after IP injection** 5.1 lid1c Many of our mouse models are inducible, meaning that upon tamoxifen injection we can delete a gene ('Floxed' genes) or reinstate gene expression ('Floxed-Stop'). This requires 5 IP injections with Tamoxifen. A typical experiment with inducible alleles has 4 groups (Mutant, Wildtype with and without CRE, all receiving Tamoxifen). We expect that in the coming 5 years we will submit 5 study plans to charachterize mice injected with Tamoxifen using electrophysiology. 5.1 lid1c We expect that in the coming 5 years we will submit 15 study plans to charachterize mice injected with Tamoxifen using molecular biology. 5.1 lid1c **V. Animals for tissue collection after ICV injection with tool compound** 5.1 lid1c To manipulate the expression of target genes within our GA lines we will typical 5.1 lid1c Such experiments typically has 4 groups (Mutant, Wildtype with and without tool compound). For ICV injection we need to take into account 10-15% drop-out mice (eaten pups or incorrectly injected animals). Hence, groupsize becomes 14 for electrophysiology and 7 for molecular biology experiments. 5.1 lid1c
Gender	Most phenotypes do not differ between male and female patients and mice, and hence we will use both sexes. In the event there is a gender bias, we will choose the gender with the strongest phenotype.
Genetic alterations	For our research, it is vital that we use mouse models that carry mutations similar to those encountered in patients giving the models a high construct validity which in turn translates to a high face validity.
Strain	We will use normal (non-GA) control and genetically altered (GA) mice in different standard genetic backgrounds (eg Bl6, 129 and FVB) or combinations (eg F1 Bl6 and 129) thereof depending on how the genetic background affects the phenotypes we see in the strains.

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

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

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

The discomfort depends on how the tool compounds are administered. This can be through IP, SC or IV injections without the use of anaesthesia. When mice are injected ICV or intracerebrally, hypothermia-anaesthesia (P0-P3) is applied. For implantation of an osmotic pump (1x) or administration via intrathecal or intracerebroventriuclar (ICV) injection or via a cannula, the procedure is carried out only once/mouse and requires surgery with adequate general anaesthesia and perioperative analgesia.

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

Tool compounds can potentially have unforeseen side effects. Also, it is possible that the animals do not recover well from surgery after ICV injections. Also, complications such as infections at the site of injection may occur.
From our experience, most genetic modifications themselves have no effect on the well-being of the animal, and phenotypic changes are only observed through advanced experimental testing where specific phenotypic modifications can be detected. Most GA animals will not have a harmful phenotype. However, we can potentially expect a mild harmful phenotype such as epilepsy when we cross two mutants, and this has been accounted for in our calculations. It is difficult to predict beforehand the severity of the phenotype we expect as this can differ between humans and mice. In some cases where harmful phenotypes are expected, the generation of inducible models may be the solution.

Explain why these effects may emerge.

Some of the new lines have not been characterised and although the impact of most procedures on animal welfare are well known, the effect of the tool compounds are unknown and may elicit side effects.

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

If unexpected adverse effects are observed, immediate action will be taken. E.g., the drug dose will be reduced or treatment may be withdrawn altogether. If appropriate we will administer pain medication or remove the animal from the experiment and euthanise it.

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

All mice will be observed daily with respect to several parameters (overall appearance, size, confirmation and growth, coat condition, behaviour, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013

Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:

1. Stunted growth (>15% reduced weight compared to littermates).
2. Moderate circulatory or respiratory problems.
3. The development of a clinical neurological disease that results in sustained suffering (eg they display deviant behaviour such as abnormal aggression, ataxia, lethargy or epilepsy).

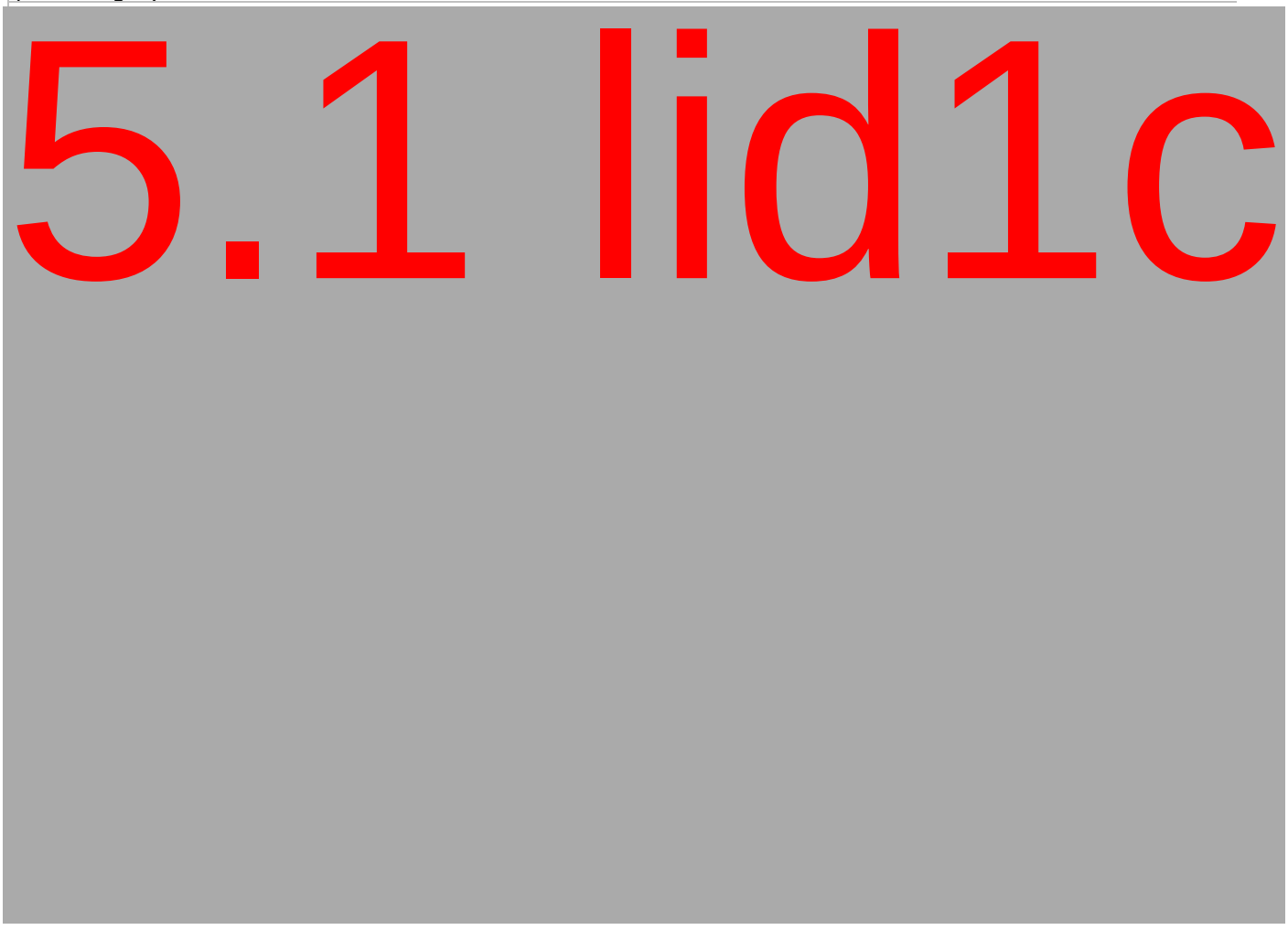
For animals that underwent IP or ICV injection of tool compounds, we will also humanely kill the animal if two days after the start of the experiment there is a decrease in body weight of maximally 20%, with a recovery within 2 days post-surgery to a max of 10% (relative to their weight at the start of the experiment, or for juvenile mice, compared to untreated littermates), or if mice experience any discomfort during the entire experiment such that the cumulative discomfort would rise beyond "mild".

Indicate the likely incidence.

Less than 1%: We have extensive experience with generating new GA lines in the last 20 years, which include all our current Ube3a related models including both the large deletion AS model as well as the large duplication (Dup15q) mouse model. So far, none of these lines show any harmful phenotype and vary rarely do we have to kill an animal because of the above criteria. Since including an individual animal with even mild discomfort can affect the measurement outcome, animals are likely to be killed well before the above criteria are met.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).



To manipulate the expression of inducible alleles, mice may receive (max 5) IP Tamoxifen injections prior to sacrificing (Severity: Mild).

To manipulate the expression of other target genes, mice may receive a single ICV injection with tool compound under anaesthesia prior to sacrificing (Severity: Moderate).

G. 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	Most experiments are performed using cell culture that requires no animals. When we use tool compounds to manipulate gene expression, they are first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when a tool compound is found to function well in in vitro and ex-vivo experiments, will it be tested in vivo, which reduces the number of animals we use.
Reduction	Before we embark on choosing the genes for generating novel GA lines, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation. We also employ some mice for *in utero* manipulation of the brain to determine whether this causes a phenotype. Once we decide to test a therapeutic, this is first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when the therapeutic is found to function well in in vitro and ex-vivo experiments, will it be tested in vivo, which reduces the number of animals we use.
Refinement	The lab has obtained extensive experience in the last 2 decades in the type of procedures been described and knows how to determine when mice are approaching humane end points such as weight loss, trembling, fur condition etc. The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of neurodevelopmental disorders, and participate in scientific meetings. Also we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.
Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals
If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.
Animals are only killed for scientific reasons or to resolve welfare concerns.

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 3 — Type of animal procedure: Behavioural analysis of mice.

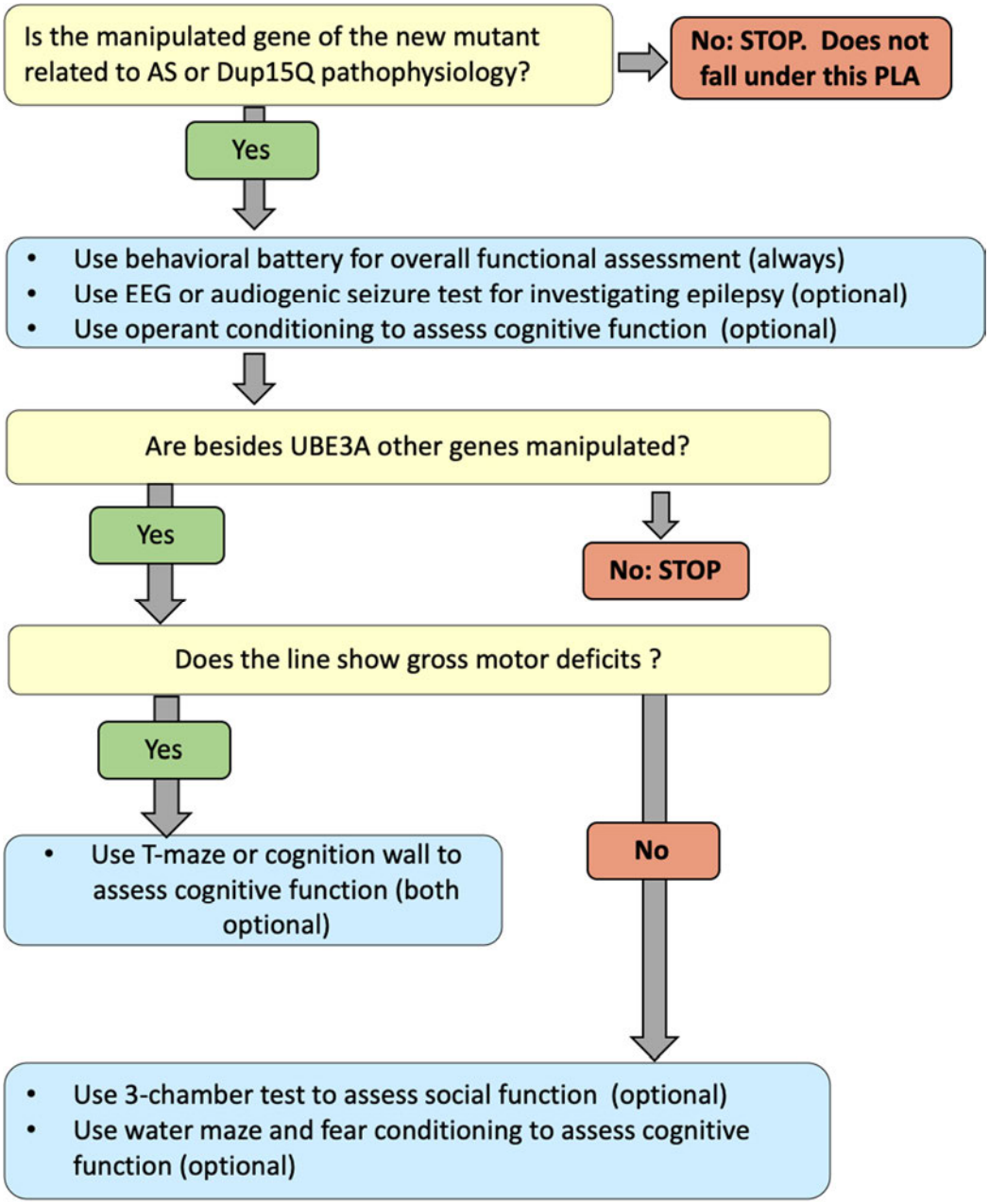
2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

Behavioral change is the ultimate read-out of our brain. Mouse models for neurodevelopmental disorders often recapitulate the main features: **intellectual disability, abnormal behaviour, motor deficits, sleep deficits, abnormal EEG and epilepsy**. This research concerns the behavioural analyses performed on existing or newly generated (**Appendix 1**) AS/Dup15q mouse models using behavioural tests. This appendix includes the standardized behavioural test battery that was optimized and implemented in the lab to efficiently and objectively assess phenotypes as observed in AS mice. It also includes additional tests that may yield a phenotypic difference between WT and mutant littermates. New and existing mouse models will be subjected to these tests to establish whether or not phenotypic differences are observed and can be used as a functional assay to determine the effect of genetic, therapeutic or pharmacological intervention in the future. We also want to test additional paradigms to be able to detect novel phenotypic differences between GA and WT mice. These experiments are needed to address **aim 1** (role of cellular UBE3A in brain development), **aim 2** (synergy between UBE3A and neighbouring genes) and **aim 3** (identify brain regions affected by loss of UBE3A).

For **all aims,** we may administer Tamoxifen to induce expression of Floxed alleles. 5.1 lid1c



Strategy to characterize the new mouse lines

The rational to use each test is explained below. 5.1 lid2h

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

A). A highly standardized behavioral test battery for gross motor performance and innate behaviours, for which we have shown that it is sensitive to the loss of UBE3A. This tests battery is now widely used in the AS field. Mice that solely have a UBE3A manipulation will only be run in this test battery.

We have set up a behavioural test battery which probes motor issues, and a number of innate behaviours such as anxiety issues and behaviours that are often affected in AS mouse models. For the tests described below, a single cohort of (treated) mice, comprising of mutant and WT control mice, will be subjected to a maximum of 7 tasks for which we have demonstrated a difference in performance between WT and mutant mice. After extensive handling of the mice to decrease stress and to get the animals acquainted with the experimenters, all mice in the cohort are sequentially subjected to these tasks over a period of 2-4

weeks. The most stressful task (5.1 lid1c (highest discomfort score) is done last, so as to not interfere with the outcome of the earlier tests. After the behavioural testing, the mice are humanely killed to analyse the brain tissue. The animals are carefully acquainted to handling before they testing ensues to minimize stress levels. The animals are randomly selected to be tested and the handler/observer (tester) is blinded for the specific genotype or treatment. With the exception of the 5.1 lid1c all tests are considered mild discomfort, but the sum of these tests result in a cumulative discomfort of moderate.

The battery has the following tests and is performed in this order:

Hind limb clasping reflex (max 10s, twice)(Severity: mild). Hind limb clasping is a marker of motor impairment and cerebellar dysfunction. The clasping test evaluates the animal's hind limb response during tail suspension 10 cm above the home cage. If the hind limbs are splayed out- ward, away from the abdomen, the mouse is assigned a score of 0. If one hind limb is retracted toward the abdomen, the animal receives a score of 1. If both hind limbs are partially retracted towards the abdomen, it receives a score of 2. The animal receives a score of 3 if the animal's hind limbs are entirely retracted and touching the abdomen.

Wire hang test (max. 60s once) (Severity: mild). The Wire hang test is used to measure subacute muscle function and fatigue. A horizontal wire (2 mm in diameter, 40 cm in length) is suspended 20 cm above a padded table. The animal is positioned to cling in the middle of the wire with its forepaws for one 60 s trial, and latency to fall is recorded.

Accelerating Rotarod or Accelerating Reverse Rotarod (2 trials of max. 5 min a day, for max. 5 days) (Severity: mild). This test measures motor coordination/performance. Mice are tested 2 times a day (ITI 1hour) on the Rotarod (a turning accelerating cylinder with a diameter of 3 cm (accelerated 4-40 rpm). This is repeated over a total of 5 days. The outcome parameter is the amount of time the mice can stay on the rod without falling. Maximum time per trial is 5 min. The reverse rotarod is the same as the accelerating Rotarod with the exception that the mice are placed on the rotating drum in the opposite direction, forcing them to walk backwards. This is a more demanding task than the regular accelerating Rotarod, and depending on the severity of the motor impairments we choose either one of these rotarod paradigms.

Open field (max. 10 min, once) (Severity: mild). This test measures anxiety and general activity. Mice are placed individually in a brightly lit 120 cm diameter circular open field for a period of 10 minutes and their exploratory behaviour is recorded on video and analysed by tracking software.

Marble burying (max. 30 min once) (Severity: mild). This test measures anxiety/repetitive behaviour. Mice are placed for 30 min. in a clean polycarbonate cage with a rich amount of bedding material. On top of the bedding material 20 blue glass marbles are arranged in an equidistant 5 x 4 grid and the animals are given access to the marbles for 30 minutes. Most (WT) mice will try to bury the marbles. The outcome is the number of marbles buried (visually inspected).

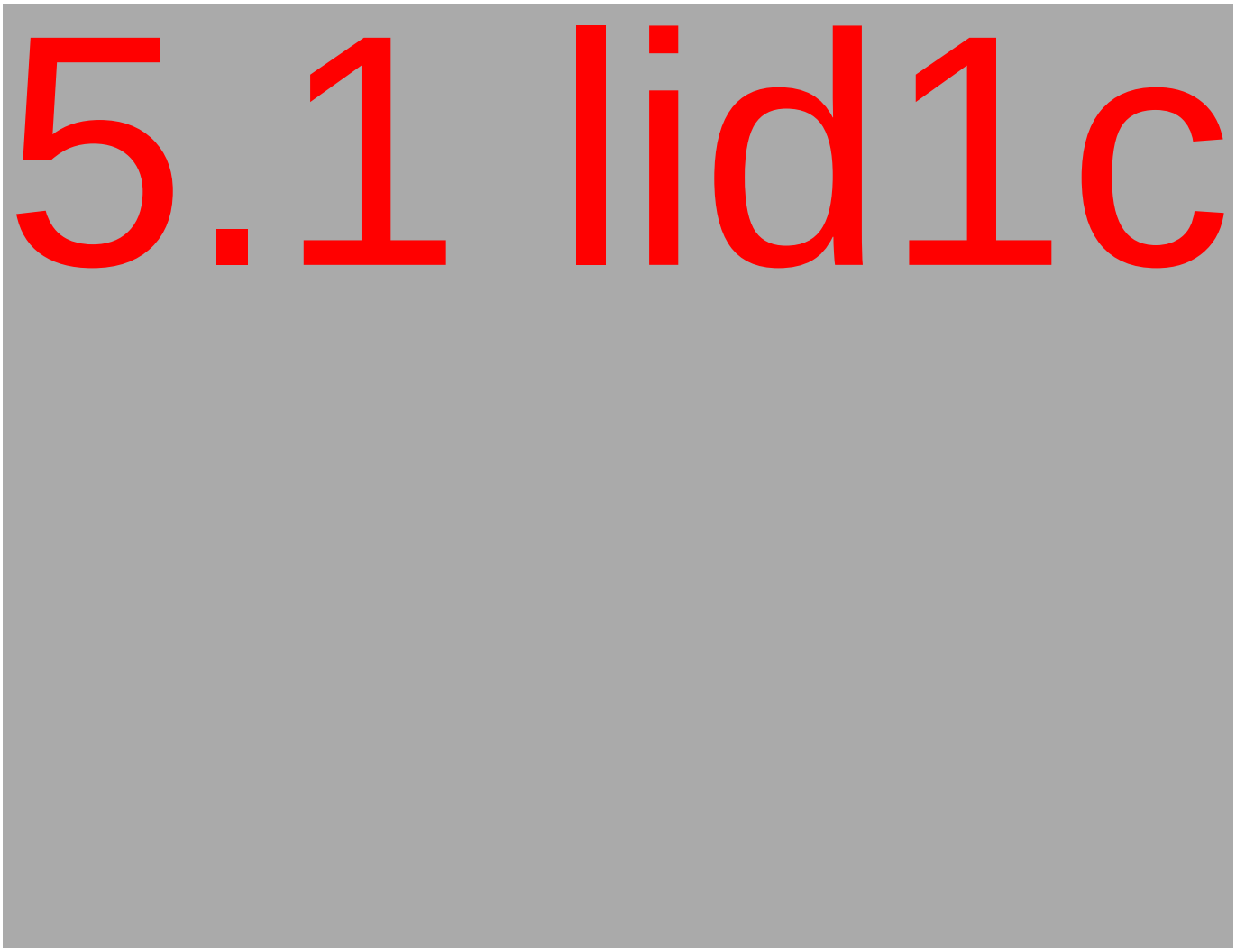
Nest building (max. 7 days continuous) (Severity: mild). This test examines the nest building behaviour of a singly housed mouse over a period of maximally 7 days. On day 1 of the test 3 cotton squares (nestlets) are added to the cage to be used by the mouse to build a nest. The weight of the remaining unused nestlets is determined each morning for maximally 7 days, allowing for the quantification of the nest building behaviour.

For novel mouse models, we may extend the characterisation with the following tests (each test requires a distinct cohort of animals and all tests are classified as moderate):

B. Cognitive tests:
These tests are very important to study whether deletion or duplication of other genes in the locus affects cognitive function. All tests described below require a **separate cohort** of mice. All these tests are classified as moderate.

Morris Water maze (max. 2 times daily for 1 min; for 14 days) The water maze test is a very sensitive test to probe spatial learning deficits as a measure of hippocampal function. As such, it is generally considered the gold standard.. In order to reduce the stress levels, a week prior to the start mice will be handled every day for 2-5 minutes. Handling consists of picking up the mouse and putting it on the hand or sleeve. Adaptation will show by more relaxed behaviour and some exploratory initiatives. This reduces the stress levels of the animals during the water maze test and consequently reduces the number of mice that have to be excluded from this test because of underperformance. During training, the mice will receive 2 trials a day for 60 sec to find the hidden platform just beneath the surface of a pool (diameter 1.20 meters) filled with visually opaque warm water (temp 25-26 degrees Celsius). Visual cues, such as coloured shapes or patterns, are placed around the pool in plain sight of the animal. After 1 min of swimming has passed the mouse will be placed on the platform for 30 sec. This will be repeated 5-14 days depending on the learning curve (finding the platform). At the end of the training, the mice will get a probe trial in which the platform is removed to see if the mouse has learned the location of the platform. Various parameters are measured including average distance to platform, latency to escape, path length, and velocity.

T-maze (max. 10 times 1 min; for 7 days): A T-maze is a simple maze used in animal cognition experiments. It is shaped like the letter T (or Y), providing the mouse with a straightforward choice. T-mazes are used to study rodent learning and flexibility when the task is modified. The animals are briefly (overnight) food deprived prior to the test and learn the test through positive reinforcement (food rewards). They can enter and leave both arms and the learning outcome is the immediate proper choice between the arms.



5.1 lid1c

C. Tests designed to study social deficits:

These tests are very important to study the role of a gene/protein in autism. If the mutation in question is linked in humans to autism, GA mice carrying the mutation may be subjected to these tests in an effort to determine the effect of treatment in reversing autism phenotype. All tests described below require a **separate cohort** of mice.

Three chamber test: (max. 1 hour; daily for 5 days). This test measures how much a test mouse interacts with a stimulus mouse (known or unknown) as compared to interaction with an object. The test mouse is placed in the centre compartment (43x40 cm) of the three-chamber set-up, and is monitored to explore the outer compartments containing either an object or a confined stimulus mouse.

D. Tests measuring brain activity and epilepsy: Also for both the 5.1 lid1c and audiogenic seizure tests, a separate cohort of mice are needed

These test are important to study epilepsy, which is a serious clinical complication for Angelman syndrome and Dup15q syndrome. We consider these tests as moderate discomfort.

5.1 lid1c

Sensitivity to Audiogenic seizures (Max 20s twice): To determine the susceptibility of mice to audiogenic seizures, both mutant and WT littermates are subjected to the noise made by vigorously scraping scissors across the metal grating of a cage lid, which generates a 100dB noise. This is done for 20 seconds, or less if a tonic-clonic seizure develops before that time. A typical seizure lasts 5-15s, and the animals appear fully recovered within 30 seconds. This test is done at the start of an experiment (baseline) and if needed repeated one more time after a specific treatment.

E. Introduction of novel testing paradigms

It is possible that during these 5 years we may introduce a test that is not described here for testing AS mice. In particular in the domain of social tests (autism) and cognitive tests (learning disability) there is a need for better (more robust, less intense) tests. Such test will not exceed the discomfort of the tests described here, and will only be considered if it provides a clear scientific advance or is better with respect to reduction or refinement. For instance, we are interested in exploring the value of video based recordings to determine seizure frequency, which would reduce or eliminate the need of invasive EEG recordings.

Administration of tamoxifen or tool compounds to modulate gene expressions

Prior to behavior, animals may be subjected to drugs that activate/inactivate the gene/allele (induction). For example, the animals may receive maximally 5 IP injections of tamoxifen (maximum volume of 5ul/g) to induce brain-specific gene deletion/activation in the case of floxed alleles. Animals may also be subjected to administration of the 5.1 lid1c

administration of these compounds will never exceed moderate discomfort.

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

5.1 lid1c [redacted] is indicated how many mice are needed for each behavioural test. In our study a power of 0.80 will be used, considering some unexpected variation we will use n=15. This provides us with sufficient statistical power to run a 1- or 2-way ANOVA and detect meaningful effect sizes. We typically do not consider phenotypes to be robust if more than 15 animals are required. On the other hand, we do not go below 10 animals per group because it limits our statistical power and analysis, and becomes too vulnerable for outliers. Experience has also helped us determine the number of mice need to run a well powered EEG experiment. We need 12 mice per genotype to detect meaningful effect sizes for each dose of therapeutic.For each new mutant mouse model, the tasks that will be employed will be based on what is known in the literature or expected, based on the nature of the mutation and gene involved. The presence of a performance difference between WT and mutant mice will first need to be verified for each individual task, and the robustness of the performance difference will determine the group size.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice GA	N/A (Annex I species specifically bred)	Pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	Mus musculus: genetically modified and wild type littermates in different standard genetic backgrounds (eg Bl6, 129) or combinations (eg F1 Bl6 and 129) thereof. We choose this species based on the fact that they are genetically similar to humans and because we are able to test relevant parameters (eg. motor learning, cognitive tests, epilepsy etc) in mice that are often translatable to humans

Origin	All animals are bred in the institute or come from a registered supplier ensuring the animals are genetically pure and free of known infectious agents. The mice are socially housed, whenever possible, under the same standard conditions unless the behavioural test does not allow for this (eg. Nest building test). In those situations, the mice are single-housed for as short a period as possible.
Life stages	All behavioral tests are performed on adult mice. However, when behavior is combined with ICV injection of tool compounds, P1 or >P21 pups may be used for ICV injection.
Number	As explained earlier in the "experimental approach", from extensive experience gathered over the past 15 years, we have determined that for **a typical behavioural test we need a group size of** 5.1 lid1c This provides us with sufficient statistical power to run a 1 or 2-way ANOVA and detect meaningful effect sizes. Smaller effect sizes can be detected by larger numbers, but the biological relevance of such small effects is often questioned. Conversely, given the variability of behavioural testing, smaller group sizes can readily result in Type I or Type II errors. For **audiogenic seizures and** 5.1 lid1c we use a group size of 5.1 lid1c **The number of groups** within an experiment is dependent on the complexity of the genotypes. For Cre lines as well ICV injection we typically have 4 groups (WT/ Mutant with and without Cre or ICV injection). Although double mutants may yield 8 groups of more, we maximally use 6 groups to perform behavior. A larger group size would inflate the statistical power and is practically not manageable. 5.1 lid1c Below we estimate the maximum number of mice we will use in the coming 5 years. For each experiment the number of mice will be detailed and justified in the study plan. **I. Behavioral analysis without additional procedures or discomfort** 5.1 lid1c To characterize our recently developed mouse models and future mouse models we will perform behavioural analysis on mice with different genotypes to perform one of the following: 1. The behavioural test-battery (single cohort) 2. Tests to measure Cognitive deficits (one cohort per test) 3. Tests to measure Social deficits (one cohort per test) 4. Seizure tests (one cohort per test) 5. Novel tests (one cohort per test) We expect that in the coming 5 years we will submit max. 5.1 lid1c We expect that in the coming 5 years we will submit 5.1 lid1c **II. Behavioral analysis with mild discomfort** 5.1 lid1c It is conceivable that one of our lines shows mild discomfort or that mild discomfort arises when mutants are intercrossed. We are in particular alert for epilepsy, as we see this in both AS as well as Dup15 patients. For such mice, good quantification of seizures is needed. If the mice show motor deficits they can't be used for most of our tests, but we still need testing to assess the severity of the motor deficits. We expect that in the coming 5 years we will submit max. 5.1 lid1c

	We expect that in the coming 5 years we will submit 5.1 lid1c **III. Behavioral analysis upon IP injection** 5.1 lid1c For our inducible lines we will use IP injection of Tamoxifen to activate Cre mediated gene expression. We expect that in the coming 10 years we will submit 5.1 lid1c We expect that in the coming 5 years we will submit max. 5.1 lid1c **IV. Behavioral analysis upon ICV injection** 5.1 lid1c 5.1 lid1c For ICV injection we need to take into account 10-15% drop- out mice (eaten pups or incorrectly injected animals), which inflates the 5.1 lid1c We expect that in the coming 5 years we will submit 5.1 lid1c We expect that in the coming 5 years we will submit max. 5.1 lid1c
Gender	Most phenotypes do not differ between male and female patients. Hence we will use both sexes, but will make sure that sexes are balanced between genotypes and treatments. In the unlikely event there is a strong sex bias, we will choose the sex with the strongest phenotype.
Genetic alterations	For our research, it is vital that we use mouse models that carry mutations similar to those encountered in patients, giving the models a high construct validity which in turn translates to a high face validity.
Strain	Different standard genetic backgrounds (eg Bl6, 129) or combinations thereof (eg F1 Bl6 and 129) are used due to the sensitivity of the test paradigm to genetic background. For example the majority of our behavioural assays are carried out in a Bl6/129 background as a phenotypic difference is seen in these mice and not in pure Bl6 mice.

C. Accommodation and care
Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?
☐ Yes
☒ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices
All mice are housed and cared for in accordance with Annex III. Below are the exceptions: In a number of tests, animals will be single housed. This applies to: Nest building: These mice are single housed for a period of maximum 7 days.

5.1 lid1c

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5.1 lid1c

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

The discomfort depends on the test and whether or not the mouse received ICV injection or EEG surgery. For P0-P3 ICV injections, hypothermia-anaesthesia is used.

Surgical procedures involving P>21 mice (ICV and EEG pedestal placement) will be carried out under general anaesthesia with adequate peri and postoperative analgesia.

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

Whenever possible, mice are group-housed but some tasks, such as the nest building task, requires the mice to be single housed. The order of tasks will be planned in such a way as to minimise the length of time the mice are single housed.

For tasks that 5.1 lid1c

Behavioural experiments will be carried out in the light period. This will have some effect on animal welfare, but this does not significantly add to the cumulative harm of the described tests.

With the exception of a few tasks, we do not expect the procedures to compromise the welfare of the animal beyond the distress resulting from the test. However, some of the animals are treated with a compound that could potentially have unforeseen side effects. Also, it is possible that the animals do not recover well from surgery after ICV injection.

Most GA animals will not have a harmful phenotype, but we can expect epilepsy, although this has thus far never been seen in our AS/Dup15q lines.

Explain why these effects may emerge.

This is expected to be a rare situation since the impacts of most procedures are well known, however, the therapeutics implemented are new and may elicit side effects.

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

If unexpected adverse effects are observed, immediate action will be taken. E.g., the drug dose will be reduced or treatment may be withdrawn altogether. If appropriate we will administrate pain medication or remove the animal from the experiment and euthanise it.

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

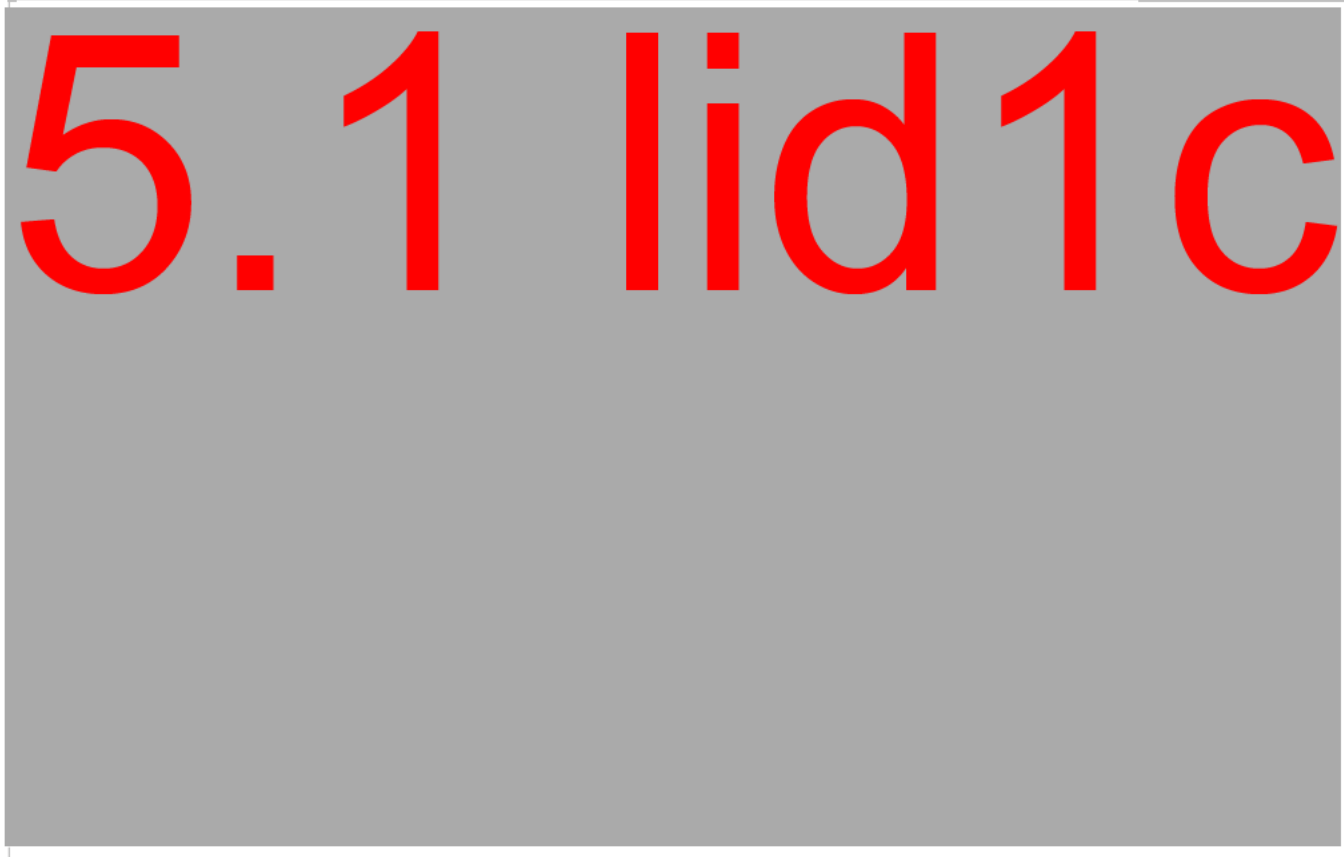
Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:
1. The animal stops eating or drinking.
2. Decrease of body weight of 10% relative to their weight at the start of the experiment, stunted growth before onset of the experiment (15% reduced compared to littermates).
3. For the 5.1 lid1c task, when a reduction in body weight of 20% or more occurs, the animals are removed from the experiment and fed ad libitum. If bodyweight decreases further the animals will be humanely killed.
4. Moderate circulatory or respiratory problems.
5. The development of a clinical neurological disease that results in sustained suffering beyond mild discomfort. If this involves seizures, we will terminate the animal when more than 10 seizures a day shorter than 30 seconds, or 2 seizures lasting more than 2 minutes per day, we will discontinue breeding that line. Also, we will terminate the mice if >20 seizures/day are observed in a seizure study.

Indicate the likely incidence.

According 5.1 lid2h, it is very rare to observe these events. We estimate this to be less than 1%.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).



Cumulative discomfort will never exceed moderate

Mice GA

5.1 lid1c

G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to study the effect of the genetic modification on brain development and brain function. To date, studying GA mice is the only reliable method to investigate the function of a gene in brain development and brain function *in vivo*.
Reduction	Before we embark on behavioral studies, we perform molecular and or electrophysiological studies. Only when these studies have resulted in significant interesting outcomes, we will initiate behavioral studies. This reduces the number of animals. When we use tool compounds to manipulate gene expression, they are first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when a tool compound is found to function well in in vitro and ex-vivo experiments, it will be tested in vivo, which reduces the number of animals we use. In addition, we perform molecular studies (which require less animals and are less invasive) before we decide to do behavioral experiments.
Refinement	The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of Angelman syndrome, and participate in scientific meetings. Also we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

All mice will be killed at the end of the experiment to allow for tissue isolation and analysis.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia. This causes least pain and stress for the animals

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons or to resolve welfare concerns

NIET-TECHNISCHE PROJECTSAMENVATTING

Naam van het project	Mechanismen die ten grondslag liggen aan Angelman Syndroom
NTS-identificatiecode	NTS-NL-842742 v.1
Nationale identificatiecode van de NTS *Veld wordt niet gepubliceerd.*	
Land	Nederland
Taal	nl
Indiening bij EU *Veld wordt niet gepubliceerd.*	ja
Duur van het project, uitgedrukt in maanden.	60
Trefwoorden	Angelman syndroom Muismodellen verstandelijk handicap DUP15Q
Doel(en) van het project	Fundamenteel onderzoek: Zenuwstelsel

DOELSTELLINGEN EN VERWACHTE VOORDELEN VAN HET PROJECT

Beschrijf de doelstellingen van het project (bijvoorbeeld het aanpakken van bepaalde wetenschappelijke onduidelijkheden, of wetenschappelijke of klinische behoeften).	Angelman syndroom (AS) en Dup15q syndroom zijn ernstige neurologische aandoeningen die bij ongeveer 1: 20.000 geboorten voorkomt. Deze aandoeningen resulteren in een ernstige ontwikkelingsachterstand, verstandelijke handicap, motorische stoornissen, gedragsafwijkingen waaronder autisme, een verstoorde slaapcyclus, en de afwezigheid van spraak. Een groot aantal patiënten heeft epilepsie. Dup15q patiënten kunnen aan deze aanvallen overlijden. Angelman syndroom wordt voornamelijk veroorzaakt door verlies van het 15q11-13-gebied op chromosoom 15, terwijl Dup15q syndroom juist veroorzaakt wordt door een duplicatie van ditzelfde chromosomale gebied. Voor beide aandoeningen staat vast dat het UBE3A gen, dat in dit 15q11-13 gebied ligt, essentieel is voor het ontstaan van de symptomen. Dit voorstel richt zich daarom specifiek op het verkrijgen van inzicht in de rol van het UBE3A gen in de hersenontwikkeling. We kijken daarbij naar de functie van het UBE3A eiwit, en wat het effect is van geen of juist te veel UBE3A, op de ontwikkeling van de hersenen en het ontstaan van epilepsie. Ook willen we graag weten welke hersengebieden daarbij precies betrokken zijn. We kijken daarnaast ook naar de andere genen in het 15q11-13 gebied. We hopen dat de kennis die we vergaren uiteindelijk leidt tot een betere behandeling van de AS en DUP15q patiënten.
Welke potentiële voordelen kan dit project opleveren? Leg uit hoe de wetenschap vooruit kan worden geholpen of mensen, dieren of het milieu uiteindelijk voordeel kunnen hebben bij het project. Maak, waar van toepassing, een onderscheid tussen voordelen op korte termijn (binnen de looptijd van het project) en voordelen op lange termijn (die mogelijk pas worden bereikt nadat het project is afgerond).	We hopen dat ons onderzoek leidt tot een beter begrip van hoe het UBE3A eiwit functioneert en wat de effect is van veranderingen in de expressie van omliggende genen. Dit kan leiden tot nieuwe behandelingen voor kinderen met Angelman syndroom of Dup15q syndroom. We hebben eerder laten zien dat we bevindingen in muizen kunnen vertalen naar de kliniek, zoals een genetische therapie voor de behandeling van kinderen met het Angelman syndroom, die momenteel wordt uitgevoerd 5.1 lid2h .

VOORSPELDE SCHADE

In welke procedures worden de dieren gewoonlijk gebruikt (bijvoorbeeld injecties, chirurgische procedures)? Vermeld het aantal en de duur van deze procedures.	Appendix 1: Generatie en import nieuwe lijnen en fokken met ongerief 5.1 lid2h [redacted] - Fokken van muizen met een mild fenotype als gevolg van de mutatie (aantal 500) Appendix 2: Verzamelen van muizenweefsel - Opzetten van neuronale culturen met hersenen uit embryo's. Moeders worden verdoofd en gedood (aantal 200), embryo's worden uit de buik gehaald en gelijk gedood zonder behandeling. Het hersenweefsel van de embryo's wordt gebruikt (aantal 1000). - Muizen van verschillende leeftijden worden onder verdoving gedood om weefsels te verzamelen voor diverse proeven (aantal 2576). Appendix 3: Karakterisering van muizen. - Muizen worden blootgesteld aan diverse gedragstaken (2260) - Muizen worden geopereerd en voorzien van een pedestal die epilepsie registreert ('EEG' meting) (aantal 840)
Wat zijn de verwachte gevolgen/nadelige effecten voor de dieren, bijvoorbeeld pijn, gewichtsverlies, inactiviteit/verminderde mobiliteit, stress, abnormaal gedrag, en wat is de duur van die effecten?	Appendix 1: Generatie en import nieuwe lijnen en fokken met ongerief Over het algemeen hebben de muizen niet al te veel last van de behandelingen. De operaties worden gedaan onder narcose en pijnstilling en de dieren zijn binnen een paar uur weer actief. Omdat we hier te maken hebben met genetisch gemodificeerde muizen is het mogelijk dat sommige nieuwe lijnen een mild fenotype vertonen. We schatten in dat maximaal 10% van de experiment muizen hier last van heeft. Dit kan variëren van een milde motorische afwijking tot epileptische aanvallen. Appendix 2: Verzamelen van muizenweefsel Voor een aantal dieren (moeders en embryo's neuronale culturen) worden de muizen gedood zonder voorafgaande (be)handeling. De overige dieren worden behandeld via injecties of operatief behandeld onder narcose en postoperatieve pijnstilling. Appendix 3: Gedragsexperimenten. Muizen worden onderworpen aan een of meerdere gedragstaken. De meeste van deze taken zijn niet stressvol voor de dieren. Soms is individuele huisvesting nodig, wat stressvol is voor de muizen. De duur hiervan wordt zo kort mogelijk gehouden. Ook voor bepaalde taken is het nodig om de dieren te onderwerpen aan voedseldeprivatie, om ze te motiveren om voedsel als beloning te zien. Een ander voorbeeld van een stressvolle taak is de audiogene epilepsie test waarbij muizen gedurende maximaal 20 seconden blootgesteld worden aan een hard (±100dB) geluid. Bij sommige mutantmuizen lijnen leidt dit tot een epileptische aanval. Muizenlijnen waarvan verwacht wordt dat ze gevoelig zijn voor spontane epileptische aanvallen worden voorzien van een (draadloos) EEG meetinstrument. Hiermee wordt de hersenactiviteit gemeten dmv elektrodes die operatief geplaatst worden in aanwezigheid van postoperatieve pijnstilling. De hersenactiviteit wordt voor een periode van maximaal 2 weken gemeten.

Welke soorten en aantallen dieren zullen naar verwachting worden gebruikt? Wat zijn de verwachte ernstgraden en de aantallen dieren in elke ernstcategorie (per soort)?

Soort:	*Totaal aantal*	*Geraamde aantallen naar ernstgraad*			
		Terminaal	*Licht*	*Matig*	*Ernstig*
Muizen (Mus musculus)	7766	0	3916	3850	0

Wat gebeurt er met de dieren die aan het einde van de procedure in leven worden gehouden?

Soort:	*Geraamd aantal te hergebruiken, in het habitat-/houderijsysteem terug te plaatsen of voor adoptie vrij te geven dieren*		
	Hergebruikt	*Teruggeplaatst*	*Geadopteerd*

Geef de redenen voor het geplande lot van de dieren na de procedure.	Dieren worden aan het eind van het experiment gedood en weefsels worden verzameld voor biochemische, immunocytochemische en elektrofysiologische analyses.

TOEPASSING VAN DE DRIE V'S

1. Vervanging Beschrijf welke diervrije alternatieven op dit gebied voorhanden zijn en waarom zij niet voor het project kunnen worden gebruikt.	Een groot deel van onze onderzoeksvragen wordt beantwoord door gebruik te maken van neuronen uit stamcellen verkregen uit patiënten, daarvoor is dus geen dierproef voor nodig. Echter, om gedetailleerd inzicht te krijgen in functie van het UBE3A gen in de hersenontwikkeling, is het essentieel om een muismodel te gebruiken waarin dezelfde DNA-verandering aanwezig is als in de patiënt. Tot op heden is het bestuderen van genetisch gemodificeerde muizen de enige betrouwbare methode om de functie van een gen in hersenontwikkeling en hersenfunctie in levende dieren te onderzoeken.
2. Vermindering Leg uit hoe de aantallen dieren voor dit project zijn bepaald. Beschrijf de stappen die zijn genomen om het aantal te gebruiken dieren te verminderen en de beginselen die zijn gebruikt bij het opzetten van de studies. Beschrijf, waar van toepassing, de praktijken die gedurende het hele project zullen worden toegepast om het aantal dieren die in overeenstemming met de wetenschappelijke doelstellingen werden gebruikt, tot een minimum te beperken. Deze praktijken kunnen bijvoorbeeld bestaan uit proefprojecten, computermodellen, het delen van weefsel en hergebruik.	In het huidige PLA worden proeven gedaan op gekweekte cellen, waarmee we meerdere experimenten kunnen uitvoeren. Dit vermindert het aantal proefdieren. Het maximaal benodigde aantal dieren per experiment wordt met name bepaald op basis van eerdere experimenten. Om de aantallen laag te houden maken we daarbij zo veel mogelijk gebruik van testen die een maximaal verschil laten zien tussen mutant en controledieren. Ook realiseren we een vermindering van de benodigde aantal dieren door: - Zowel mannen als vrouwen te gebruiken wanneer de mutatie dit toelaat. - Een volgorde van verschillende testen met hetzelfde dier te kiezen, waarbij we de meeste informatie met het minste aantal dieren kunnen verkrijgen. - Geoptimaliseerde protocollen te gebruiken om de gevoeligheid van de test te maximaliseren met een minimale groepsgrootte. - Gebruik te maken van goed gedefinieerde en gestandaardiseerde genetische achtergronden.
3. Verfijning Geef voorbeelden van de specifieke maatregelen (bv. verscherpte monitoring, postoperatieve behandeling, pijnbestrijding, training van dieren) die in verband met de procedures moeten worden genomen om de welzijnskosten (schade) voor de dieren tot een minimum te beperken. Beschrijf de mechanismen om gedurende de looptijd van het project nieuwe verfijningstechnieken in gebruik te nemen.	We zullen eerst ex-vivo experimenten doen, en alleen wanneer die aanleiding geven tot vervolg onderzoek zullen we in vivo (gedrag) experimenten uitvoeren. Om stress verschijnselen tijdens de gedragsexperimenten te minimaliseren bij onze muizen worden de muizen: 1) zo veel mogelijk groep-gehuisvest 2) dagelijks 'gehandeld' om de dieren aan de onderzoekers gewend te laten raken. De onderzoekers blijven op de hoogte van de laatste ontwikkelingen op dit onderzoeksgebied door (inter)nationale samenwerking met andere onderzoeksgroepen die onderzoek doen naar de genetische achtergrond van neurologische ontwikkelingsstoornissen, en nemen deel aan wetenschappelijke bijeenkomsten. Ook controleren we door middel van uitgebreide literatuuronderzoeken (Medline, Pubmed) of er geen geschikte alternatieven beschikbaar zijn voor een bepaald project. Tijdens ons lopende onderzoek zal intensief literatuuronderzoek worden voortgezet op zoek naar nieuwe ontwikkelingen met betrekking tot alternatieve benaderingen die in deze studie moeten worden geïmplementeerd.
Licht de keuze van de soorten en de bijbehorende levensstadia toe	Muizen in alle stadia (vanaf embryo, pasgeborenen tot volwassenen (max. 8 maanden)) zullen worden onderworpen aan verschillende analyses om inzicht te krijgen in het ziekte mechanisme. Hierdoor kunnen we de verschillende ontwikkelingsstadia van de hersenen goed bestuderen. De gekozen leeftijd hangt daarnaast af van de functionele test. Voor gedragsexperimenten dienen de muizen minimaal 6 weken oud te zijn.

VOOR EEN BEOORDELING ACHTERAF GESELECTEERD PROJECT

Project geselecteerd voor BA?	nee
Termijn voor BA	

Reden voor de beoordeling achteraf

Bevat ernstige procedures	
Maakt gebruik van niet-menselijke primaten	
Andere reden	
Toelichting van de andere reden voor de beoordeling achteraf	

AANVULLENDE VELDEN

Nationaal veld 1 *Veld wordt niet gepubliceerd.*	
Nationaal veld 2 *Veld wordt niet gepubliceerd.*	
Nationaal veld 3 *Veld wordt niet gepubliceerd.*	
Nationaal veld 4 *Veld wordt niet gepubliceerd.*	
Nationaal veld 5 *Veld wordt niet gepubliceerd.*	
Startdatum project *Veld wordt niet gepubliceerd.*	
Einddatum project *Veld wordt niet gepubliceerd.*	
Goedkeuringsdatum project *Veld wordt niet gepubliceerd.*	
ICD-code 1 *Veld wordt niet gepubliceerd.*	
ICD-code 2 *Veld wordt niet gepubliceerd.*	
ICD-code 3 *Veld wordt niet gepubliceerd.*	
Link naar de eerdere versie van de NTS buiten het EC-systeem	

Centrale Commissie Dierproeven

Advies aan CCD

Datum 15 februari 2023
Betreft Advies Secretariaat over Aanvraag projectvergunning Dierproeven AVD202216352

Instelling:	5.1 lid2h
Onderzoeker:	5.1 lid2e
Project:	Mechanisms underlying the pathophysiology of Angelman Syndrome
Aanvraagnummer:	AVD202216352
Betreft:	Nieuwe aanvraag
Categorieën:	Fundamenteel onderzoek

1 Inzicht in aanvraag en de eventuele knelpunten en risico's
Deze aanvraag is minder diepgaand getoetst vanwege een kwalitatief goed DEC-advies.

Proces	De volgende vragen zijn gesteld aan de aanvrager: - Een deel van uw aanvraag behelst het opzetten van nieuwe genetisch gewijzigde muizenlijnen. De CCD heeft afgelopen december een nieuwe handreiking op de website gepubliceerd die vanaf 1 januari van kracht is. Kunt u uw aanvraag in lijn brengen met deze handreiking? - In bijlage 2 onder K mist de onderbouwing waarom het noodzakelijk is de dieren te doden, kunt u dit toevoegen? Over de NTS: - De NTS dient anoniem te zijn. Kunt u de verwijzing naar 5.1 lid2h 5.1 lid2h verwijderen? - Het algemeen publiek heeft geen inzicht in de verschillende bijlagen, kunt u de verwijzing hiernaar veranderen? - in de NTS komen technische termen en vakjargon voor zoals, maar niet beperkt tot, super-ovulatie, blastocysten, fenotype en founders. Kunt u de tekst nog een keer doorlopen en moeilijke termen uitleggen of vervangen door meer toegankelijke woorden? - U gebruikt de term "mild ongerief". Kunt u dit vervangen door "licht ongerief" zodat dit in lijn is met de tabel?

Overzicht van opmerkingen bij AdviesNotaCCD, d.d. 15-02-2023_met opmerkingen (1).pdf

Pagina: 1

Nummer: 1 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 20-2-2023 15:47:10 +01'00'
In bijlage 2 onder Number komt het aantal voor onderdeel I niet overeen met de optelsom. 5.1 lid1c .

Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 20-2-2023 15:50:07 +01'00'
De aantallen in bijlage 3 kloppen ook niet helemaal.

Nummer: 2 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 20-2-2023 15:49:12 +01'00'
Ook als ik de totale aantallen in de 3 bijlagen optel, kom ik 100 dieren tekort voor het aantal genoemd in de NTS.

Nummer: 3 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 20-2-2023 16:02:04 +01'00'
De DEC geeft aan (C1) dat de doelstellingen ingrijpend zijn veranderd na de bespreekrondes (3 in totaal). Kan de aanvrager zomaar de doelstellingen aanpassen tijdens het aanhouden van de DEC?

	- Kunt u iets toelichten over de aard van de gedragstaken en het ongerief dat de dieren hierdoor ondervinden?

Naam proef	Diersoort	Stam	Aantal dieren	Herkomst
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort				
	Muizen (Mus musculus)	wildtype en genetisch gemodificeerd	890	Dieren die voor onderzoek gefokt zijn
3.4.3.2. Collection of mouse tissue				
	Muizen (Mus musculus)	wildtype en genetisch gemodificeerde dieren	3.776	Dieren die voor onderzoek gefokt zijn
3.4.3.3. Behavioural analysis of mice.				
	Muizen (Mus musculus)	genetisch gemodificeerde dieren	3.100	Dieren die voor onderzoek gefokt zijn

Gebruik van mannelijke en vrouwelijke dieren

3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt.

3.4.3.2. Collection of mouse tissue

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt. citaat: Most phenotypes do not differ between male and female patients and mice, and hence we will use both sexes. In the event there is a gender bias, we will choose the gender with the strongest phenotype.

3.4.3.3. Behavioural analysis of mice.

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt. Zie 3.4.3.2.

Locatie uitvoering experimenten	- Alle proeven vinden plaats in een instelling van een vergunninghouder. - Er zijn geen problemen bekend met de vergunninghouder.

2 DEC advies

DEC-advies	Citaten uit het DEC advies: C10 (huisvesting): In principe zullen alle dieren conform de eisen in bijlage III van richtlijn 2010/63/EU worden gehuisvest. De studs en de

gevasectomeerde mannelijke dieren worden individueel gehuisvest. In het kader van de wetenschappelijke experimenten zullen dieren alleen voorafgaand en gedurende een aantal gedragsexperimenten (de nestbouwtest, tijdens de voerbeperkingsperiode voorafgaand aan de cognitietesten) voor enige tijd individueel gehuisvest worden. Deze huisvesting is niet conform de richtlijn, maar is vanuit het belang van het experiment voldoende onderbouwd en in termen van ongerief geclassificeerd.
De commissie acht het uitvoeren van de gedragstesten in de lichtperiode ook een afwijking van de standaard huisvestingomstandigheden (zie ook C11).

C11 (ongerief): Bij het genereren van nieuwe lijnen is er bij de donordieren (superovulatie), de gevasectomeerde mannen en de foster dieren sprake van cumulatief matig ongerief.
Gezien de aard van de betrokken genen is het niet uitgesloten dat er bij de genetisch gemodificeerde dieren sprake zou kunnen zijn van een lijn met constitutioneel ongerief. Bij de lijnen die tot nu toe gegenereerd zijn is dit nog nooit aangetroffen. Desondanks wordt de optie open gehouden dat er bij maximaal 1 lijn voor de fok en in de experimenten gebruik gemaakt zal worden van dieren met constitutioneel maximaal gering ongerief. Dieren met een fenotype resulterend in meer dan gering ongerief (de kans hierop is zeer klein) worden direct gedood.
Ten gevolge van de toedieningen en mogelijke effecten van de toegediende stoffen (voor gen-inductie en behandeling) is er voorafgaand aan het doden voor het verzamelen van weefsel kans op gering (en in een zeer beperkt aantal gevallen (<1%) matig) ongerief.
De uitvoering van het grootste aantal gedragstesten gaan (inclusief een eventuele voerbeperking of de toediening van tamoxifen of 'tool compounds') niet gepaard met meer dan licht ongerief. Bij een aantal testen 5.1 lid1c de Morris watermaze, 5.1 lid1c en 5.1 lid1c is sprake van maximaal matig ongerief.
In een aparte groep dieren wordt door middel van audiogene prikkels de gevoeligheid voor het optreden van epileptische verschijnselen bepaald. Ook hier is sprake van matig ongerief (vooral bepaald door implantatie van de intracerebrale afleid electroden onder anesthesie).
Het optreden van spontane insulten wordt in beperkte mate verwacht en zal nooit resulteren in meer dan licht ongerief.
De commissie gaat er vanuit dat de keuze om de gedragsexperimenten uit te voeren in de licht(niet actieve) periode ook een bron van additioneel ongerief is.
De commissie heeft uitgebreid met de indieners gecorrespondeerd en met de IvD vertegenwoordiger gesproken over de situatie of en wanneer

Pagina: 3

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	er bij de voorgestelde experimenten zich situaties zouden kunnen voordoen waarbij door een stapeling van ongerief ten gevolge van verschillende interventies (elk op zich zelf resulterend in matig ongerief) uiteindelijk sprake zou kunnen zijn van cumulatief ernstig ongerief. De conclusie hieruit was dat dit in geen enkel geval te verwachten is, gezien de temporele scheiding tussen de handelingen. Op basis hiervan is de commissie tot de conclusie gekomen dat het aangegeven ongerief (3916 dieren licht en 3850 matig) realistisch, voldoende onderbouwd en herleidbaar is ingeschat en geclassificeerd. C18 (geslachten): In de wetenschappelijke geïnitieerde experimenten wordt gebruik gemaakt van zowel mannelijke als vrouwelijke dieren. In het (onwaarschijnlijke) geval dat er een duidelijk sekse gebonden verschil in fenotype wordt gevonden zullen experimenten uitgevoerd worden in het geslacht met het sterkste fenotype. Deze situatie wordt niet verwacht. C20 (doden om niet wetenschappelijke redenen): Alle dieren in de wetenschappelijk geïnitieerde experimenten worden gedood om wetenschappelijke redenen (postmortem analyse van weefsel (vooral hersenen). Voor de studs en gevasectomeerde mannelijke dieren is sprake van continued use. De draagmoeders worden gedood in het kader van het project, hoewel niet om wetenschappelijke of welzijnsredenen. Dit is moreel problematisch, maar zeer moeilijk te vermijden omdat er geen wetenschappelijke of andere bestemming is voor deze dieren. Ethische afweging van de DEC: 1) Rechtvaardigt het belang van het verkrijgen van wetenschappelijk inzicht in de rol van het UBE3A gen en het 15q11-13 gencluster bij de normale en afwijkende hersenontwikkeling waarbij ook aangrijpingspunten voor mogelijk therapeutische interventies zouden kunnen worden geïdentificeerd, het gebruik van 7766 (deels genetisch gemodificeerde) muizen en de hiermee gepaard gaande aantasting van hun integriteit en welzijn (licht ongerief (3916 muizen), matig ongerief (3850 muizen)? 2) Bij alle dieren (7766 muizen) is sprake van een aantasting van integriteit en welzijn. Bij maximaal 3850 muizen is er risico op matig ongerief en bij 3916 muizen is er sprake van licht ongerief. Het risico op het bereiken van een humaan eindpunt wordt zeer klein geschat. Het voorgestelde onderzoek beoogt inzicht te verkrijgen in de rol van het UBE3A gen en het 15q11-13 gencluster tijdens de normale en verstoorde hersenontwikkeling en het identificeren van het neuronale substraat

hiervan.
Naast dit onmiskenbaar fundamenteel wetenschappelijk belang is voor de groep patiënten met genetisch modificaties in het UBE3A gen (en hun naaste familie en de maatschappij) het belang groot.
Hoewel dat buiten de directe scope van dit project valt zou het beschikken over adequate behandelingen voor patiënten met afwijkingen in het UBE3A gen van groot persoonlijk en maatschappelijk belang zijn.

5.1 lid2h

5.1 lid2h is het uitvoeren van fundamenteel wetenschappelijk onderzoek in dit onderzoeksveld van groot belang. Niet in het minst voor de snelle vertaling van de wetenschappelijke resultaten naar klinische trials en uiteindelijk toepassing in de kliniek.

3) De DEC is overtuigd dat de in dit project voorgestelde experimenten een bijdrage zullen leveren aan de kennis over de rol van het UBE3A gen op de hersenontwikkeling en het neuronale substraat dat hierbij betrokken is.
Naast dit onmiskenbaar grote fundamenteel wetenschappelijke belang zijn er uiteindelijk verschillende maatschappelijke belangen gediend met de resultaten van dit onderzoek. In eerste instantie het belang van de patiënten met afwijkingen in hun UBE3A gen. Het mogelijk reduceren van hun ziektelast dient naast een groot persoonlijke belang voor de betreffende patiënten ook een aanzienlijk breder maatschappelijk belang: de verminderde ziektelast bij de familie, de zorg en de maatschappij als geheel. Omdat voor UBE3A gen gerelateerde type ziektebeelden op dit moment nog geen therapie beschikbaar is, is uiteindelijk dit onderzoek ook voor de betrokken clinici van groot belang.
De DEC is van mening dat de directe belangen voor onderzoekers en de wetenschap en de mogelijke uiteindelijke belangen voor de betreffende patiënten, hun directe omgeving, de berokken clinici en de maatschappij, voldoende zwaar wegen om het schaden van de belangen van de proefdieren om gevrijwaard te blijven van een aantasting van hun integriteit en een licht of matige aantasting van hun welzijn te rechtvaardigen.
De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de directe doelstellingen binnen de looptijd van het project. De brede (klinische) inbedding van het onderzoek maakt de drempel naar eventueel verder klinisch onderzoek en uiteindelijk toepassing van de verkregen kennis in de kliniek zeer laag.
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 voorkomen zal worden dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven. De DEC is van oordeel dat de hier boven geschetste belangen de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van aantasting van hun integriteit en in een deel van de dieren matig ongerief rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen de aantasting van integriteit en ongerief dat de dieren wordt berokkend, is in dat opzicht voldaan. Het DEC advies is Positief Het uitgebrachte advies is niet gebaseerd op consensus. Het uitgebrachte advies is met een meerderheid van stemmen tot stand gekomen. Er is sprake van een minderheidsstandpunt wat zich primair richt op de belangen van de belanghebbende 'de wetenschap' (in termen van kwaliteit) en de daarmee verbonden belanghebbende 'de proefdieren' (in termen van aantallen en ongerief en aantasting van integriteit). Dit minderheidsstandpunt had in algemene zin niet betrekking op het belang van fundamenteel onderzoek naar het Angelman en Dup15q syndroom, maar was meer gericht op het open karakter van de aanvraag (de startmomenten van het onderzoek, de inherente onzekerheden met betrekking tot de keuzes voor bepaalde modellen en de vrijheid bij de keuzes in het kader van minimalisatie van de aantallen dieren en het ongerief bij het opzetten van de experimenten). Daarnaast was er de opvatting dat de belangen van de proefdieren geschaad worden door niet onderbouwd rekening te houden met de aangetoonde welzijnsconsequenties bij het uitvoeren van de gedragstesten tijdens de rustperiode. De meerderheid van de commissie heeft in haar afweging betrokken dat onzekerheid (zeker op een termijn van 5 jaar) inherent is aan de uitvoering van fundamenteel wetenschappelijk onderzoek en heeft bij het hierboven aangegeven 'open karakter van het project' ook zwaar de aantoonbare ervaring en kwaliteit van de wetenschappelijke infrastructuur binnen de onderzoeksgroep en de instelling en de kwaliteit van de in het verleden verkregen resultaten (bijvoorbeeld in de vorm van publicaties in high ranking tijdschriften en translaties naar de kliniek) meegewogen. Voor wat betreft de uitvoering van de gedragsexperimenten in de lichtperiode. Dit betreft een zeer algemene en breed toegepaste en

	geaccepteerde werkwijze. De commissie sluit niet uit dat deze werkwijze (dieren in een voor hen biologisch gezien 'vreemde' situatie plaatsen) onderdeel is van het model. In de correspondentie met de indieners, haar afweging en in haar advies heeft de commissie aangeven dat deze werkwijze resulteert in additioneel ongerief en (mogelijk ook) in een aantasting van de integriteit. (zie C11 en C12).

3 Kwaliteit DEC advies

Kwaliteit DEC-advies	
Het DEC advies is helder en volledig. Er is inzicht gegeven in de vragen die gesteld zijn. Bij de beantwoording van de C vragen gebruikt u een heldere onderbouwing. De ethische afweging volgt op een logische manier uit de antwoorden op de C vragen. Het minderheidsstandpunt is op een duidelijke manier weergegeven.	

4 Inhoudelijke beoordeling
3V's

Er is in voldoende mate onderbouwd dat de doelstelling niet zonder dieren behaald kan worden en het project met zo min mogelijk dieren en zo verfijnd mogelijk wordt uitgevoerd.

Hergebruik	Er is geen sprake van hergebruik van dieren.

Naam proef	**Worden de dieren gedood?**	**Doden volgens richtlijn?**
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort	Ja	volgens de richtlijn.
3.4.3.2. Collection of mouse tissue	Ja	niet volgens de richtlijn. Citaat: Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals
3.4.3.3. Behavioural analysis of mice.	Ja	volgens de richtlijn.

Naam proef		
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort		
Muizen (Mus musculus)	Ongerief: 21,3% Matig 78,7% Licht	
3.4.3.2. Collection of mouse tissue		
Muizen (Mus musculus)	Ongerief: 14,8% Matig 85,2% Licht	
3.4.3.3. Behavioural analysis of mice.		
Muizen (Mus musculus)	Ongerief: 100,0% Matig	

5 Samenvatting

5.2 lid1

Het betreft een vervolg op de eerder uitgegeven vergunning AVD5.1 lid2h
Onder de voorgaande vergunning zijn functies van het UBE3A eiwit geïdentificeerd en zijn enkele muis modellen gegenereerd. Ook zijn er artikelen gepubliceerd en is er een data set openbaar gemaakt.

In principe worden de dieren gehuisvest en verzorgd volgens de richtlijn. In bijlage 3 wordt hier in bepaalde gevallen vanaf geweken. Voor de nest building test worden muizen maximaal 7 dagen individueel gehuisvest. Voor 5.1 lid1c worden dieren maximaal 8 uur gevast. Voor 5.1 lid1c 5.1 lid1c ondergaan muizen voedsel restrictie in die mate dat zij op een gewicht van 85% zullen zitten ten opzichte van het gewicht onder ad libitum voedselinname.5.1 lid1c 5.1 lid1c Daarnaast worden gedragstesten uitgevoerd in de lichtfase.[1]De DEC merkt dit ook aan als afwijking van standaard huisvestingomstandigheden en als bron van ongerief.

[2]

Er zullen mannelijke en vrouwelijke muizen worden gebruikt. Indien wordt gemerkt dat het fenotype geslachtsafhankelijk is kan worden gekozen voor gebruik van één geslacht. Deze situatie wordt echter niet verwacht.

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5.2 lid1

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Ik zou in de samenvatting en de terugkoppeling DEC ook nog iets vermelden over het minderheidsstandpunt van de DEC en waarom we daar niet in meegaan.

In bijlage 3 zullen muizen gedragstesten ondergaan waarbij schokken worden gebruikt 5.1 lid1c of waarin zij moeten zwemmen 5.1 lid1c n morris water maze). De keuze voor deze tests is voldoende onderbouwd. De DEC heeft met de aanvrager en IvD gesproken over de mogelijkheid dat door een opeenstapeling van handelingen sommige muizen ernstig ongerief zouden kunnen ervaren. De conclusie uit deze correspondentie was dat dit niet te verwachten is.

6 Voorstel besluit incl. voorstel geldigheidsduur van de vergunning

5.2 lid1

De ingangsdatum van de vergunning kan niet voor de verzenddatum van de beschikking zijn en zal indien van toepassing aangepast worden. Dit is ook het geval bij een voorgenomen besluit.

7 Concept beschikking voor akkoord CCD

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5.2 lid1

Centrale Commissie Dierproeven

Advies aan CCD

Datum 01 maart 2023
Betreft Advies Secretariaat over Aanvraag projectvergunning Dierproeven AVD202216352

Instelling:	5.1 lid2h
Onderzoeker:	5.1 lid2e
Project:	Mechanisms underlying the pathophysiology of Angelman Syndrome
Aanvraagnummer:	AVD202216352
Betreft:	Nieuwe aanvraag
Categorieën:	Fundamenteel onderzoek

1 Inzicht in aanvraag en de eventuele knelpunten en risico's
Deze aanvraag is minder diepgaand getoetst vanwege een kwalitatief goed DEC-advies.

Proces	De volgende vragen zijn gesteld aan de aanvrager: - Het totale aantal aangevraagde dieren in de bijlagen komt niet overeen met het aantal in de NTS. Kunt u de aantallen nalopen en de documenten met elkaar in overeenstemming brengen? - Een deel van uw aanvraag behelst het opzetten van nieuwe genetisch gewijzigde muizenlijnen. De CCD heeft afgelopen december een nieuwe handreiking op de website gepubliceerd die vanaf 1 januari van kracht is. Kunt u uw aanvraag in lijn brengen met deze handreiking? - In bijlage 2 lijkt de berekening voor het aantal dieren in experiment I niet te kloppen (tekst onder de tabel in sectie B). Kunt u nagaan of het aantal aangevraagde dieren klopt met de berekeningen? - In bijlage 2 onder K mist de onderbouwing waarom het noodzakelijk is de dieren te doden, kunt u dit toevoegen? Over de NTS: - De NTS dient anoniem te zijn. 5.1 lid2h - Het algemeen publiek heeft geen inzicht in de verschillende bijlagen, kunt u de verwijzing hiernaar veranderen?

	- in de NTS komen technische termen en vakjargon voor zoals, maar niet beperkt tot, super-ovulatie, blastocysten, fenotype en founders. Kunt u de tekst nog een keer doorlopen en moeilijke termen uitleggen of vervangen door meer toegankelijke woorden? - U gebruikt de term "mild ongerief". Kunt u dit vervangen door "licht ongerief" zodat dit in lijn is met de tabel? - Kunt u iets toelichten over de aard van de gedragstaken en het ongerief dat de dieren hierdoor ondervinden?

Naam proef	**Diersoort**	**Stam**	**Aantal dieren**	**Herkomst**
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort				
	Muizen (Mus musculus)	wildtype en genetisch gemodificeerd	890	Dieren die voor onderzoek gefokt zijn
3.4.3.2. Collection of mouse tissue				
	Muizen (Mus musculus)	wildtype en genetisch gemodificeerde dieren	3.776	Dieren die voor onderzoek gefokt zijn
3.4.3.3. Behavioural analysis of mice.				
	Muizen (Mus musculus)	genetisch gemodificeerde dieren	3.100	Dieren die voor onderzoek gefokt zijn

Gebruik van mannelijke en vrouwelijke dieren

3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt.

3.4.3.2. Collection of mouse tissue

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt. citaat: Most phenotypes do not differ between male and female patients and mice, and hence we will use both sexes. In the event there is a gender bias, we will choose the gender with the strongest phenotype.

3.4.3.3. Behavioural analysis of mice.

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt. Zie 3.4.3.2.

Locatie uitvoering experimenten	- Alle proeven vinden plaats in een instelling van een vergunninghouder. - Er zijn geen problemen bekend met de vergunninghouder.

2 DEC advies

DEC-advies	Citaten uit het DEC advies: C10 (huisvesting): In principe zullen alle dieren conform de eisen in bijlage III van richtlijn 2010/63/EU worden gehuisvest. De studs en de gevasectomeerde mannelijke dieren worden individueel gehuisvest. In het kader van de wetenschappelijke experimenten zullen dieren alleen voorafgaand en gedurende een aantal gedragsexperimenten (de nestbouwtest, tijdens de voerbeperkingsperiode voorafgaand aan de cognitietesten) voor enige tijd individueel gehuisvest worden. Deze huisvesting is niet conform de richtlijn, maar is vanuit het belang van het experiment voldoende onderbouwd en in termen van ongerief geclassificeerd. De commissie acht het uitvoeren van de gedragstesten in de lichtperiode ook een afwijking van de standaard huisvestingomstandigheden (zie ook C11). C11 (ongerief): Bij het genereren van nieuwe lijnen is er bij de donordieren (superovulatie), de gevasectomeerde mannen en de foster dieren sprake van cumulatief matig ongerief. Gezien de aard van de betrokken genen is het niet uitgesloten dat er bij de genetisch gemodificeerde dieren sprake zou kunnen zijn van een lijn met constitutioneel ongerief. Bij de lijnen die tot nu toe gegenereerd zijn is dit nog nooit aangetroffen. Desondanks wordt de optie open gehouden dat er bij maximaal 1 lijn voor de fok en in de experimenten gebruik gemaakt zal worden van dieren met constitutioneel maximaal gering ongerief. Dieren met een fenotype resulterend in meer dan gering ongerief (de kans hierop is zeer klein) worden direct gedood. Ten gevolge van de toedieningen en mogelijke effecten van de toegediende stoffen (voor gen-inductie en behandeling) is er voorafgaand aan het doden voor het verzamelen van weefsel kans op gering (en in een zeer beperkt aantal gevallen (<1%) matig) ongerief. De uitvoering van het grootste aantal gedragstesten gaan (inclusief een eventuele voerbeperking of de toediening van tamoxifen of 'tool compounds') niet gepaard met meer dan licht ongerief. Bij een aantal testen (de '5.1 lid1c ' , de Morris watermaze, 5.1 lid1c) is sprake van maximaal matig ongerief. In een aparte groep dieren wordt door middel van audiogene prikkels de gevoeligheid voor het optreden van epileptische verschijnselen bepaald. Ook hier is sprake van matig ongerief (vooral bepaald door implantatie van de intracerebrale afleid electroden onder anesthesie).

	Het optreden van spontane insulten wordt in beperkte mate verwacht en zal nooit resulteren in meer dan licht ongerief. De commissie gaat er vanuit dat de keuze om de gedragsexperimenten uit te voeren in de licht(niet actieve) periode ook een bron van additioneel ongerief is. De commissie heeft uitgebreid met de indieners gecorrespondeerd en met de IvD vertegenwoordiger gesproken over de situatie of en wanneer er bij de voorgestelde experimenten zich situaties zouden kunnen voordoen waarbij door een stapeling van ongerief ten gevolge van verschillende interventies (elk op zich zelf resulterend in matig ongerief) uiteindelijk sprake zou kunnen zijn van cumulatief ernstig ongerief. De conclusie hieruit was dat dit in geen enkel geval te verwachten is, gezien de temporele scheiding tussen de handelingen. Op basis hiervan is de commissie tot de conclusie gekomen dat het aangegeven ongerief (3916 dieren licht en 3850 matig) realistisch, voldoende onderbouwd en herleidbaar is ingeschat en geclassificeerd. C18 (geslachten): In de wetenschappelijke geïnitieerde experimenten wordt gebruik gemaakt van zowel mannelijke als vrouwelijke dieren. In het (onwaarschijnlijke) geval dat er een duidelijk sekse gebonden verschil in fenotype wordt gevonden zullen experimenten uitgevoerd worden in het geslacht met het sterkste fenotype. Deze situatie wordt niet verwacht. C20 (doden om niet wetenschappelijke redenen): Alle dieren in de wetenschappelijk geïnitieerde experimenten worden gedood om wetenschappelijke redenen (postmortem analyse van weefsel (vooral hersenen). Voor de studs en gevasectomeerde mannelijke dieren is sprake van continued use. De draagmoeders worden gedood in het kader van het project, hoewel niet om wetenschappelijke of welzijnsredenen. Dit is moreel problematisch, maar zeer moeilijk te vermijden omdat er geen wetenschappelijke of andere bestemming is voor deze dieren. Ethische afweging van de DEC: 1) Rechtvaardigt het belang van het verkrijgen van wetenschappelijk inzicht in de rol van het UBE3A gen en het 15q11-13 gencluster bij de normale en afwijkende hersenontwikkeling waarbij ook aangrijpingspunten voor mogelijk therapeutische interventies zouden kunnen worden geïdentificeerd, het gebruik van 7766 (deels genetisch gemodificeerde) muizen en de hiermee gepaard gaande aantasting van hun integriteit en welzijn (licht ongerief (3916 muizen), matig ongerief (3850 muizen)?

2) Bij alle dieren (7766 muizen) is sprake van een aantasting van integriteit en welzijn. Bij maximaal 3850 muizen is er risico op matig ongerief en bij 3916 muizen is er sprake van licht ongerief. Het risico op het bereiken van een humaan eindpunt wordt zeer klein geschat.
Het voorgestelde onderzoek beoogt inzicht te verkrijgen in de rol van het UBE3A gen en het 15q11-13 gencluster tijdens de normale en verstoorde hersenontwikkeling en het identificeren van het neuronale substraat hiervan.
Naast dit onmiskenbaar fundamenteel wetenschappelijk belang is voor de groep patiënten met genetisch modificaties in het UBE3A gen (en hun naaste familie en de maatschappij) het belang groot.
Hoewel dat buiten de directe scope van dit project valt zou het beschikken over adequate behandelingen voor patiënten met afwijkingen in het UBE3A gen van groot persoonlijk en maatschappelijk belang zijn.
5.1 lid2h s het uitvoeren van fundamenteel wetenschappelijk onderzoek in dit onderzoeksveld van groot belang. Niet in het minst voor de snelle vertaling van de wetenschappelijke resultaten naar klinische trials en uiteindelijk toepassing in de kliniek.

3) De DEC is overtuigd dat de in dit project voorgestelde experimenten een bijdrage zullen leveren aan de kennis over de rol van het UBE3A gen op de hersenontwikkeling en het neuronale substraat dat hierbij betrokken is.
Naast dit onmiskenbaar grote fundamenteel wetenschappelijke belang zijn er uiteindelijk verschillende maatschappelijke belangen gediend met de resultaten van dit onderzoek. In eerste instantie het belang van de patiënten met afwijkingen in hun UBE3A gen. Het mogelijk reduceren van hun ziektelast dient naast een groot persoonlijke belang voor de betreffende patiënten ook een aanzienlijk breder maatschappelijk belang: de verminderde ziektelast bij de familie, de zorg en de maatschappij als geheel. Omdat voor UBE3A gen gerelateerde type ziektebeelden op dit moment nog geen therapie beschikbaar is, is uiteindelijk dit onderzoek ook voor de betrokken clinici van groot belang.
De DEC is van mening dat de directe belangen voor onderzoekers en de wetenschap en de mogelijke uiteindelijke belangen voor de betreffende patiënten, hun directe omgeving, de berokken clinici en de maatschappij, voldoende zwaar wegen om het schaden van de belangen van de proefdieren om gevrijwaard te blijven van een aantasting van hun integriteit en een licht of matige aantasting van hun welzijn te rechtvaardigen.
De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, dat het project goed is opgezet, en dat de gekozen strategie

	en experimentele aanpak kunnen leiden tot het behalen van de directe doelstellingen binnen de looptijd van het project. De brede (klinische) inbedding van het onderzoek maakt de drempel naar eventueel verder klinisch onderzoek en uiteindelijk toepassing van de verkregen kennis in de kliniek zeer laag. 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 voorkomen zal worden dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven. De DEC is van oordeel dat de hier boven geschetste belangen de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van aantasting van hun integriteit en in een deel van de dieren matig ongerief rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen de aantasting van integriteit en ongerief dat de dieren wordt berokkend, is in dat opzicht voldaan. Het DEC advies is Positief Het uitgebrachte advies is niet gebaseerd op consensus. Het uitgebrachte advies is met een meerderheid van stemmen tot stand gekomen. Er is sprake van een minderheidsstandpunt wat zich primair richt op de belangen van de belanghebbende 'de wetenschap' (in termen van kwaliteit) en de daarmee verbonden belanghebbende 'de proefdieren' (in termen van aantallen en ongerief en aantasting van integriteit). Dit minderheidsstandpunt had in algemene zin niet betrekking op het belang van fundamenteel onderzoek naar het Angelman en Dup15q syndroom, maar was meer gericht op het open karakter van de aanvraag (de startmomenten van het onderzoek, de inherente onzekerheden met betrekking tot de keuzes voor bepaalde modellen en de vrijheid bij de keuzes in het kader van minimalisatie van de aantallen dieren en het ongerief bij het opzetten van de experimenten). Daarnaast was er de opvatting dat de belangen van de proefdieren geschaad worden door niet onderbouwd rekening te houden met de aangetoonde welzijnsconsequenties bij het uitvoeren van de gedragstesten tijdens de rustperiode. De meerderheid van de commissie heeft in haar afweging betrokken dat onzekerheid (zeker op een termijn van 5 jaar) inherent is aan de uitvoering van fundamenteel wetenschappelijk onderzoek en heeft bij het hierboven aangegeven 'open karakter van het project' ook zwaar de aantoonbare ervaring en kwaliteit van de wetenschappelijke

	infrastructuur binnen de onderzoeksgroep en de instelling en de kwaliteit van de in het verleden verkregen resultaten (bijvoorbeeld in de vorm van publicaties in high ranking tijdschriften en translaties naar de kliniek) meegewogen. Voor wat betreft de uitvoering van de gedragsexperimenten in de lichtperiode. Dit betreft een zeer algemene en breed toegepaste en geaccepteerde werkwijze. De commissie sluit niet uit dat deze werkwijze (dieren in een voor hen biologisch gezien 'vreemde' situatie plaatsen) onderdeel is van het model. In de correspondentie met de indieners, haar afweging en in haar advies heeft de commissie aangeven dat deze werkwijze resulteert in additioneel ongerief en (mogelijk ook) in een aantasting van de integriteit. (zie C11 en C12).

3 Kwaliteit DEC advies

Kwaliteit DEC-advies	
Het DEC advies is helder en volledig. Er is inzicht gegeven in de vragen die gesteld zijn. Bij de beantwoording van de C vragen gebruikt u een heldere onderbouwing. De ethische afweging volgt op een logische manier uit de antwoorden op de C vragen. Het minderheidsstandpunt is op een duidelijke manier weergegeven.	

4 Inhoudelijke beoordeling

3V's

Er is in voldoende mate onderbouwd dat de doelstelling niet zonder dieren behaald kan worden en het project met zo min mogelijk dieren en zo verfijnd mogelijk wordt uitgevoerd.

Hergebruik	Er is geen sprake van hergebruik van dieren.

Naam proef	**Worden de dieren gedood?**	**Doden volgens richtlijn?**
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort	Ja	volgens de richtlijn.
3.4.3.2. Collection of mouse tissue	Ja	niet volgens de richtlijn. Citaat: Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals
3.4.3.3. Behavioural analysis of mice.	Ja	volgens de richtlijn.

Naam proef		
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort		
Muizen (Mus musculus)	Ongerief: 21,3% Matig 78,7% Licht	
3.4.3.2. Collection of mouse tissue		
Muizen (Mus musculus)	Ongerief: 14,8% Matig 85,2% Licht	
3.4.3.3. Behavioural analysis of mice.		
Muizen (Mus musculus)	Ongerief: 100,0% Matig	

5 Samenvatting
Er is voldoende informatie over het belang van het onderzoek, de strategie, de 3V's, de humane eindpunten en het ongerief om tot een oordeel te komen. Het DEC advies kan hieraan ten grondslag liggen.

Het betreft een vervolg op de eerder uitgegeven vergunning AVD5.1 lid2h. Onder de voorgaande vergunning zijn functies van het UBE3A eiwit geïdentificeerd en zijn enkele muis modellen gegenereerd. Ook zijn er artikelen gepubliceerd en is er een data set openbaar gemaakt.

In principe worden de dieren gehuisvest en verzorgd volgens de richtlijn. In bijlage 3 wordt hier in bepaalde gevallen vanaf geweken. Voor de nest building test worden muizen maximaal 7 dagen individueel gehuisvest. Voor 5.1 lid1c worden dieren maximaal 8 uur gevast. Voor de 5.1 lid1c ondergaan muizen voedsel restrictie in die mate dat zij op een gewicht van 85% zullen zitten ten opzichte van het gewicht onder ad libitum voedselinname. 5.2 lid1 Daarnaast worden gedragstesten uitgevoerd in de lichtfase. De DEC merkt dit ook aan als afwijking van standaard huisvestingsomstandigheden en als bron van ongerief.

Er zullen mannelijke en vrouwelijke muizen worden gebruikt. Indien wordt gemerkt dat het fenotype geslachtsafhankelijk is kan worden gekozen voor

gebruik van één geslacht. Deze situatie wordt echter niet verwacht.

In bijlage 3 zullen muizen gedragstesten ondergaan waarbij schokken worden gebruikt 5.1 lid1c of waarin zij moeten zwemmen 5.1 lid1c en morris water maze). De keuze voor deze tests is voldoende onderbouwd. De DEC heeft met de aanvrager en IvD gesproken over de mogelijkheid dat door een opeenstapeling van handelingen sommige muizen ernstig ongerief zouden kunnen ervaren. De conclusie uit deze correspondentie was dat dit niet te verwachten is.

6 Voorstel besluit incl. voorstel geldigheidsduur van de vergunning

5.1 lid1c

De ingangsdatum van de vergunning kan niet voor de verzenddatum van de beschikking zijn en zal indien van toepassing aangepast worden. Dit is ook het geval bij een voorgenomen besluit.

7 Concept beschikking voor akkoord CCD

Van: info@zbo-ccd.nl
Verzonden: woensdag 1 maart 2023 16:55
Aan: 5.1 lid2h 5.1 lid2e
CC: 5.1 lid2e
Onderwerp: Aanhouden AVD5.1 lid2h 202216352

Geachte 5.1 lid2e

Op 19-08-2022 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Mechanisms underlying the pathophysiology of Angelman Syndrome" met aanvraagnummer AVD5.1 lid2h 202216352. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In dit bericht leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

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

Niet technische samenvatting
- De NTS dient anoniem te zijn. Kunt u de verwijzing naar het 5.1 lid2h verwijderen?

- Het algemeen publiek heeft geen inzicht in de verschillende bijlagen, kunt u de verwijzing hiernaar verwijderen?

- in de NTS komen technische termen en vakjargon voor zoals, maar niet beperkt tot, super-ovulatie, blastocysten, fenotype en founders. Kunt u de tekst nog een keer doorlopen en moeilijke termen uitleggen of vervangen door meer toegankelijke woorden?

- U gebruikt de term "mild ongerief". Kunt u dit vervangen door "licht ongerief" zodat dit in lijn is met de tabel?

- Kunt u iets toelichten over de aard van de gedragstaken?

Onduidelijkheden
- Het totale aantal aangevraagde dieren in de bijlagen komt niet overeen met het aantal in de NTS. Kunt u de aantallen nalopen en de documenten met elkaar in overeenstemming brengen?

- Een deel van uw aanvraag behelst het opzetten van nieuwe genetisch gewijzigde muizenlijnen. De CCD heeft afgelopen december een nieuwe handreiking op de website gepubliceerd die vanaf 1 januari van kracht is. Kunt u uw aanvraag in lijn brengen met deze handreiking?

- In bijlage 2 lijkt de berekening voor het aantal dieren in experiment I niet te kloppen (tekst onder de tabel in sectie B). Kunt u nagaan of het aantal aangevraagde dieren klopt met de berekeningen?

- In bijlage 2 onder K mist de onderbouwing waarom het noodzakelijk is de dieren te doden, kunt u dit toevoegen?

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 dit bericht op. U kunt dit aanleveren via NetFTP.

Uw aanvraag zal 10 maart in de CCD vergadering worden besproken. Antwoorden die voor die datum zijn ingediend zullen worden meegenomen in de bespreking van uw aanvraag.

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.

Mocht u vragen hebben, dan kunt u uiteraard contact met ons opnemen.

Met vriendelijke groet,
Namens de Centrale Commissie Dierproeven

5.1 lid2e
www.centralecommissiedierproeven.nl

.....
Postbus 93118 | 2509 AC | Den Haag
.....
T: 0800 789 0789
E: info@zbo-ccd.nl

Ref: AVD5.1 lid2h02216352 (Mechanisms underlying the pathophysiology of Angelman Syndrome)

Geachte CCD-commissieleden,

Als reactie op uw vragen hebben we enkele aanpassingen aangebracht. Hieronder geven we puntsgewijs een reactie op alle vragen.

1) De NTS dient anoniem te zijn. Kunt u de verwijzing naar 5.1 lid2h verwijderen?

We hebben de verwijzing verwijderd.

2) Het algemeen publiek heeft geen inzicht in de verschillende bijlagen, kunt u de verwijzing hiernaar verwijderen?

Ook deze verwijzingen zijn verwijderd.

3) In de NTS komen technische termen en vakjargon voor zoals, maar niet beperkt tot, super-ovulatie, blastocysten, fenotype en founders. Kunt u de tekst nog een keer doorlopen en moeilijke termen uitleggen of vervangen door meer toegankelijke woorden?

De tekst is nu aangepast en daardoor toegankelijker geworden.

4) U gebruikt de term "mild ongerief". Kunt u dit vervangen door "licht ongerief" zodat dit in lijn is met de tabel?

Excuses hiervoor, dit is nu aangepast

5) Kunt u iets toelichten over de aard van de gedragstaken?

We hebben nu de gedragstaken beter omschreven:
Muizen worden blootgesteld aan diverse gedragstaken (2260), zoals het laten lopen op een roterend loopwiel, het vermogen om een nest te maken van papier; het begraven van knikkers; het meten van exploratief gedrag in een open arena; de aanwezigheid van bepaalde reflexen en de gevoeligheid om een epileptische aanval te krijgen na blootstelling aan een hard geluid gedurende 20 seconden.

6) Het totale aantal aangevraagde dieren in de bijlagen komt niet overeen met het aantal in de NTS. Kunt u de aantallen nalopen en de documenten met elkaar in overeenstemming brengen?

De getallen komen nu overeen met elkaar

7) Een deel van uw aanvraag behelst het opzetten van nieuwe genetisch gewijzigde muizenlijnen. De CCD heeft afgelopen december een nieuwe handreiking op de website gepubliceerd die vanaf 1 januari van kracht is. Kunt u uw aanvraag in lijn brengen met deze handreiking?

Het 5.1 lid2h *is aan het inventariseren om voor zover als mogelijk een generieke* 5.1 lid2h *-brede projectvergunningaanvraag voor te bereiden om in lijn met de herziene handreiking genetisch gewijzigde dieren te blijven. Hiermee wordt het aantal amendementen op bestaande, toekomstige en lopende projectvergunningen beperkt.*

We hebben toegevoegd (appendix 1): 'Initial welfare Assessment: We plan to generate and import new GA mouse lines during the next 5 years. In line with the recently revised guideline for genetically modified animals, a welfare assessment will be carried out to monitor and determine the welfare of new transgenic animals (2 generations, 7 males and 7 female control and GA mice). These animals will most likely be added to a new general project licens. If that license is not in place in time, we will write an addendum'.

8) In bijlage 2 lijkt de berekening voor het aantal dieren in experiment I niet te kloppen (tekst onder de tabel in sectie B). Kunt u nagaan of het aantal aangevraagde dieren klopt met de berekeningen?

We hebben de tekst aangepast om duidelijker te maken dat de 200 moeders die nodig zijn om de embryo's te verkrijgen gedood worden tijdens het experiment en dus meetellen bij de aantallen die in de tabel staan.
Het volgende is toegevoegd: "The dams will be sacrificed and embryo's extracted to setup the neuronal cultures. Total: 200 dams + 1000 embryos = 1200 animals"

9) In bijlage 2 onder K mist de onderbouwing waarom het noodzakelijk is de dieren te doden, kunt u dit toevoegen?

Dit ontbrak nog. We hebben het volgende toegevoegd: "The procedures of this appendix are aimed at obtaining brain tissue, which requires killing the animal." En bij de vervolgvraag: *"Mice will be sacrificed to obtain the tissues used for subsequent analyses."*

We hopen hiermee alle vragen te hebben beantwoord.

Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	Provide the title of the project.	Mechanisms underlying the pathophysiology of Angelman and Dup15q syndrome

2 Categories

2.1	Please tick each of the following boxes that applies to your project. Let op! De verplichte bijlagen verschillen per categorie. Op hetInvloket.nl leest u meer informatie over de verplichte bijlagen per	☒ Basic research ☐ Translational or applied research ☐ Regulatory use or routine production ☐ Research into environmental protection in the interest of human or animal ☐ 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 2.1.

The UBE3A gene plays an important role in a number of neurodevelopmental disorders, namely Angelman syndrome (AS; caused by loss of UBE3A)) and 15q11-q13 duplication syndrome (Dup15q syndrome; caused by duplication of a number of genes including UBE3A), as well as rare cases of autism spectrum disorders (ASD; caused by duplication of UBE3A or gain of function mutations of UBE3A).

AS is a severe neurodevelopmental disorder affecting approximately 1:20,000 births resulting in severe developmental delay (max. developmental age is ±2 years), intellectual disability, motor dysfunction, behavioural abnormalities including autism and impaired sleep cycle, and absence of speech. A large number of patients (80%) also suffer from epilepsy. AS is caused by mutations affecting the maternal copy of the UBE3A gene, which encodes a ubiquitin ligase that plays an important role in protein homeostasis in the cell. UBE3A fulfils its function by interacting with other proteins (targets) and attaching ubiquitin peptides to these targets, which can, amongst other things, mark the proteins for degradation.

To understand more about the basic biology underlying the pathophysiology of AS, we have carried out work under PLA AVD5.1 lid2h to identify the function of specific parts of the UBE3A protein, identify its target proteins and understand its role in neuronal function and brain development. We published work that showed the importance of correct localisation of the UBE3A protein to the nucleus, a property of UBE3A that is shared between mice and humans [1][2]. We found that the majority of UBE3A missense mutations as observed in patients rendered the nuclear UBE3A protein cytosolic which was enough to cause AS [3]. To study the specific role of each of the nuclear or cytoplasmic UBE3A protein isoforms, we have successfully generated a number of mouse lines in the previous 5 years that express only the nuclear or cytoplasmic UBE3A isoforms. In the coming 5 years we hope to use these mouse models to help us understand the role of each isoform. Being a ubiquitin ligase, one of the questions that still linger concerns the target proteins ubiquitinated by UBE3A. A major data set was created in a collaborative effect between our lab and the pharmaceutical industry, in which targets of UBE3A were found in a large scale spatiotemporal proteomic analysis [4]. This valuable data set is publicly available (https://www.angelman-proteome-project.org).

Despite these advancements, a lot is still unknown about the function of UBE3A in the different cellular compartments, and with which targets UBE3A interacts. We also do not know what makes UBE3A so essential to brain development. Additionally, the majority of AS patients carry a deletion that not only includes the entire UBE3A gene also but many other genes, present at the 15q11-13 locus. These patients present with a more severe phenotype making it important to disentangle the contribution of these additional genes within the AS pathophysiology.

Through imprinting, the UBE3A protein levels are tightly regulated. The importance of this tight regulation is apparent from rare patients that have multiple copies of UBE3A and suffer from autism and other neuropsychiatric disorders. Moreover, individuals carrying multiple copies of maternal 15q11.2-q13.1, the very same region that is deleted in most AS patients, suffer from Dup15q syndrome (1:5,000). Dup15q syndrome is in most cases a very debilitating neurodevelopmental condition, also characterized by intellectual disability, impaired motor coordination, autism spectrum disorder and epilepsy. The epilepsy in these patients can be severe and sometimes results in unexpected death (SUDEP). The penetrance and severity of the symptoms of Dup15q is driven by two factors: the number of duplications and whether the duplicated region is derived from the maternal chromosome or not. The symptoms are worst upon maternal inheritance of the duplications. The duplicated region includes ***UBE3A***, the only gene within this region that is expressed solely from the maternal allele in mature neurons. Although this would implicate UBE3A as the main causative factor, patients with duplications of only ***UBE3A***, as opposed to the entire 15q11.2-q13.1 area, do not show intellectual disability and epilepsy. Recently, we extended these clinical observations by showing that overexpressing UBE3A in mice also does not cause any major phenotypes, suggesting that there is a synergy between UBE3A and other gene products present in the duplicated region that underlies the Dup15q phenotypes.

There are currently no effective treatments available for AS and Dup15q and patients need life-long care. A better understanding of the fundamental biology and physiology underlying disorders involving UBE3A such as Angelman Syndrome, Dup15q and UBE3A-associated ASD can open up new directions for therapeutic strategies. These are important questions that we will address in the category 'Basic Research'. The category 'Basic research' encompasses all studies concerning the pathophysiology of the absence or overexpression of UBE3A and the role of neighbouring genes at the 15q11-13 locus as well as UBE3A target genes.

We have previously developed a5.1 lid2h [5][6], which makes this a very powerful tool for both drug testing as well as understanding the role of UBE3A in the brain. Work on mice carried out under PLA AVD5.1 lid2h has led to a publication in which we showed reversal of AS phenotypes treated with antisense oligonucleotides (ASOs) [7]. This was a proof-of-concept study that formed the foundation upon which the clinical trials are based that are currently performed on AS individuals at our center. Our lab holds AS mouse models that are already used for drug

testing on a regular basis. Importantly, our close connection to 5.1 lid2h [redacted] for both AS and Dup15q, enables us to translate preclinical findings of promising therapies to the clinic

3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The immediate goal of this project is to understand the role of UBE3A in brain development, with the ultimate goal to identify a treatment for Angelman syndrome and Dup15q syndrome. More specifically we have the following goals:

Aim 1: Investigate the cellular role of UBE3A in brain development.

The critical targets that are ubiquitinated by UBE3A ('UBE3A targets') are still poorly understood, and their identification remains a central part of our research. We have become very intrigued by our (unpublished) findings that UBE3A binds to the proteasome and could be a regulator of the proteasome. The proteasome is important for protein homeostasis as it rids the cell of aberrantly folded or excess proteins. Hence, if UBE3A is important for proteasome function, loss of UBE3A will likely affect many proteins. In this aim we will in particular study the genes/proteins that are regulated by UBE3A. Most of these experiments are performed in cultured cells, but some questions can only be addressed in neurons (ex vivo) or in living animals (behavior).

5.1 lid2h [redacted] Understanding the biological basis of UBE3A function in the neuron will be the first step in devising new therapeutic strategies.

Aim 2: Study the synergy between UBE3A and the neighbouring genes in the 15q11-13 gene cluster in both AS and Dup15q.

Although specific mutations only affecting the UBE3A gene result in AS, the majority (80%) of the patients harbour a large deletion affecting maternal chromosome 15, encompassing the 15q11-13 region (±6Mb) which includes UBE3A. The symptoms experienced by these patients are typically more severe compared to individuals carrying mutations only affecting the maternal copy of UBE3A, in particular with respect to epilepsy and their cognitive abilities. 5.1 lid1c [redacted]

The same region that is deleted in AS is present in multiple copies in Dup15q syndrome. As explained in 3.1, 5.1 lid2h [redacted]

Aim 3: Identify brain regions that are affected by loss of UBE3A

Understanding which brain regions are affected by loss (or duplication) of UBE3A is important for two reasons: (a) Once we know which brain regions underlie the mouse behavioural deficits (for instance Ube3a mice have notable marble burying deficits), we can better translate these measurements to clinical trials. (b) There is a great interest in applying genetic treatments using viral vector approaches to

target specific brain areas. But which brain areas do we need to target with these vectors? And which brain areas contribute to the limited critical treatment period in which we can obtain a full behavioural rescue. In this aim, we will address these questions. These questions can only be addressed in animal models.

3.2.2 Provide a justification for the project's feasibility.

5.1 lid2h, 5.1 lid1c [redacted]

The feasibility of this project is further demonstrated by past performance. For AVD 5.1 lid2h [redacted] we used approximately 5.1 lid2h [redacted] and 5.1 lid2h [redacted] (Appendix 1), described a robust 5.1 lid2h [redacted] (Appendix 3) which is now used in labs all over the world, and we have significantly contributed to our understanding of Angelman Syndrome (Appendix 2). 5.1 lid2h [redacted] is made available online as a searchable tool. 5.1 lid2h [redacted] Appendices 2 and 3).

With >25 publications related to AS in the last 10 years including high-profile journals, we are considered to be leaders in this field. Our average relative impact citation score is 6, meaning that our papers are 6 times more cited than the average paper in the field of neurodevelopment.

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

☒ No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

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

Social relevance: Angelman syndrome and Dup15q syndrome are very severe neurodevelopmental syndromes with an incidence of respectively 1:20,000 and 1:5,000 characterized by severe intellectual disability, intractable epilepsy, and behavioural problems, despite normal life expectancy. Hence the patients need life-long care. Little is known about the underlying pathophysiology, and currently there is no effective treatment. Even anti-epileptic drugs are often not effective in these patients, and the behavioural problems, absence of a regular day-night time sleep rhythm, as well as the inability to talk, puts a great burden on the families. Therefore, drugs that alleviate (some) of these problems are very welcome. For the identification of future therapies, we need to have a mechanistic understanding of the function of UBE3A in brain development.

'Scientific relevance:

Aim 1: Although 88 genes involved in the Ubiquitin-Proteasome-System (UPS) are causally associated with neurodevelopmental disorders, we still have a poor understanding why mutations in these genes affect the brain so strongly while apparently not giving any problems in other organs of the body. It is likely that the UPS in the brain plays a very important part in the dynamic protein homeostasis required for synaptic plasticity. While there are over 600 E3 ligases, UBE3A (also known as E6-AP) is the prototypic HECT E3 type ligase (HECT stands 'Homologous to the E6-AP Carboxyl Terminus)' and is among the most studied E3 ligases. UBE3A is also a rather unique ligase since it binds tightly to the proteasome, a feature that is observed in only 3 out of the 600 E3 ligases. Also, the notion that UBE3A exerts its function in the nucleus, rather than the synapse, has really sparked the interest of the UPS and

neuroscience field. Almost nothing is known about the role and regulation of the UPS in the neuronal nucleus.

Aim 2: In addition to understanding the role of the UBE3A protein within the cell/brain region, a large gap in our knowledge concerns the role of the other genes that are also affected in large deletion/duplication patients. This includes the HERC2 gene, which encodes an E3 ligase that is known to interact with the UBE3A protein. Understanding such interactions, both direct and indirect, will provide us with important biological insight in UBE3A functioning, and ultimately how this impacts the severity of the AS and Dup15q condition

Aim3: Knowing which brain areas are affected by loss (or duplication) of UBE3a is not only of clinical interest but also of great interest from a behavioural neuroscience point of view. The strong phenotypes in our UBE3A mice along with the many tools to induce or reduce gene expression at great spatial and temporal resolution gives us a unique handle to probe which brain areas are involved in commonly used behavioural tests.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

Laboratory animals: The goal of this application is to request the use of animals to help us understand the biology behind AS and to develop drugs for patients. The animals obviously have a negative interest, as their integrity can be affected by genetic modification in addition to applying drugs and performing surgeries and tests, all potentially harmful to the animal. The animals are eventually killed as part of the experiment. The welfare of the mice will be at most moderately affected during our experiments and it is our moral duty to ensure that the discomfort the animals endure is kept to a minimum.

Patients and parents: Patients (and indirectly their family members) with AS/Dup15q will hopefully profit from our experiments. Despite this clear positive interest, they may also need to provide cells (negative interest), often in the form of a vial of blood, from which iPSC derived cells will be generated. In addition, there are no guarantees that we succeed in identifying a treatment, and even if we do so they carry a significant risk when these therapies are applied to the patients. This risk is reduced but not excluded upon in vivo testing in animals.

The lab: The lab has a scientific interest in deciphering the aetiology of AS and Dup15q Syndrome. The results obtained from our research are not only beneficial to patients, but also to the scientific community at large as it helps us to understand UBE3A in addition to its interacting proteins/factors and the other proteins encoded by the neighbouring genes. The lab also has a strong scientific interest in understanding the role of the proteasome in the neuronal nucleus (which is an entirely unexplored topic) and understanding which brain areas contribute to commonly observed mouse phenotypes. The lab generates its income from its publications, as they will facilitate funding of new projects. With respect to current lab funding sources, 40% comes from government funding, 40% from patient organisations and 20% from industry.

Society: In terms of the societal impact, AS is a debilitating disorder that has a great impact on the afflicted individuals but certainly also on the care-givers. A treatment for some of these disorders may have a positive impact on the patient and thus on our society at large, but could also influence health costs.
Industry: some of the drugs will be developed together with the pharmaceutical industry. They will financially profit from our studies but it will increase the chance of developing a drug and get it approved for clinical trials.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

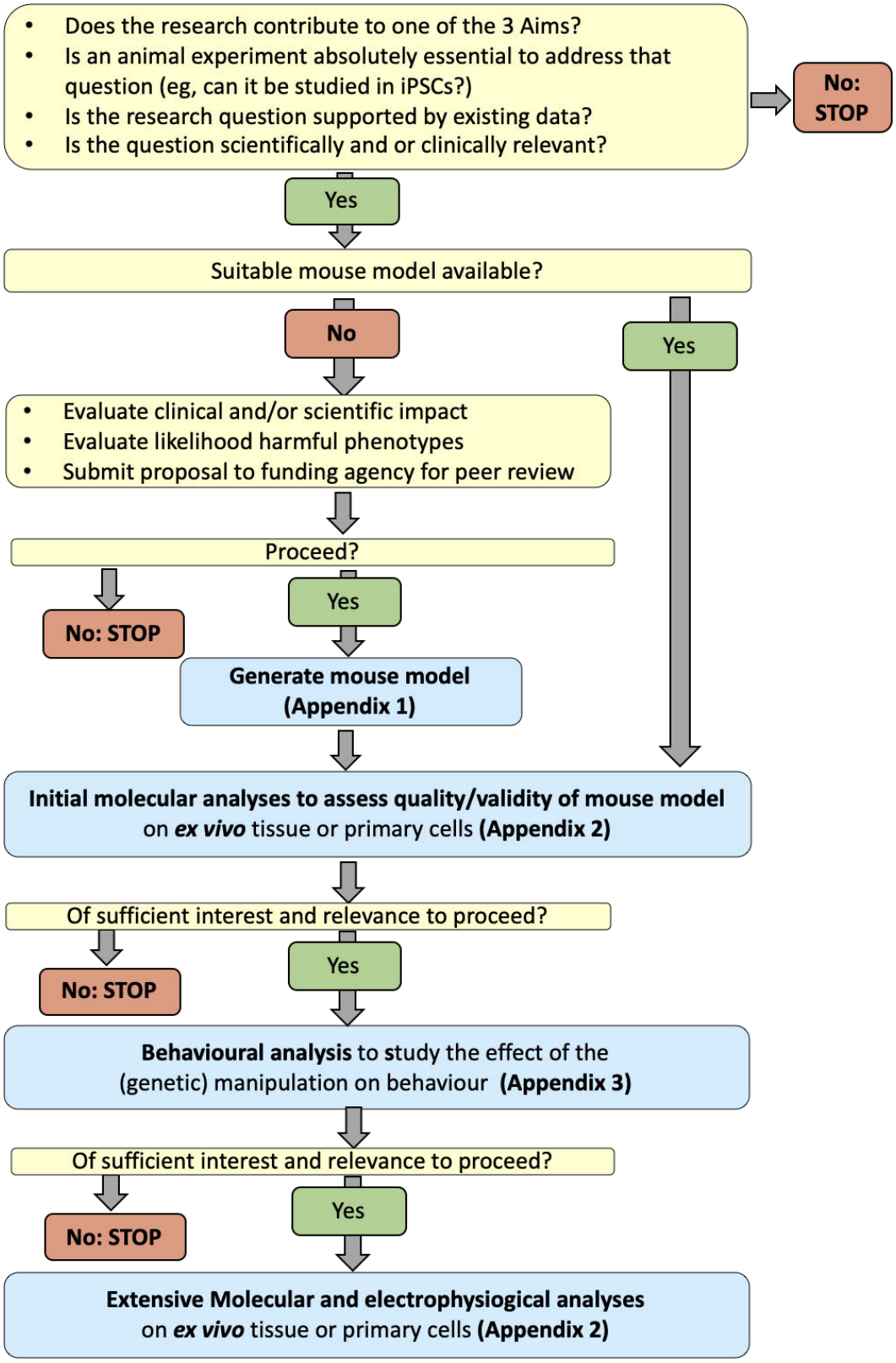

Figure 1: Overall strategy

Our research focuses on the pathophysiology (and function) of UBE3A and its synergy with genes in the 15q11-13 gene cluster , and its role in the various brain regions (see 3.2.1 Aims 1-3). Where possible our lab primarily utilises molecular and cellular studies **that do not require animals to address these questions**. Since the UBE3A-related disorders are all disorders of the brain, we focus our work specifically on neurons (including inducible pluripotent stem cells (iPSCs)). However, since UBE3A is critical for early postnatal brain development, the loss/overexpression of UBE3A and other genes in the 15q11-13 gene cluster cannot be faithfully modelled in a dish. Hence, we still need animals for certain research questions.

For this PLA we will primarily focus on characterizing the following (already available) mouse lines:

With the exception of the first line, all these mice require crosses with other lines 5.1 lid1c or Transgenic Cre driver lines) to address scientifically relevant questions. These crossings would yield far more possibilities than we can (and want to) investigate in 5 years. Also, the number of experiments that we can do is strongly dependent on the nature of the experiment. Hence, we will have to make choices based on the scientific relevance, data that we acquired, and on data that others published during this PLA. Finally, funding agencies will ultimately have a critical voice in what we actually can do. Together, these factors make it difficult to compile a complete list of experiments to be carried out in the next 5 years. The numbers listed in the appendixes are therefore based on a realistic prediction of what we can and will do in the next 5 years, based on previous experiences. In terms of discomfort, there is no a priori difference in which strain we are characterizing.

To address our research questions we will use the lines above to perform the following mouse experiments:

A. Generation of mouse models (Optional; Appendix 1): In order to study the mechanisms underlying AS and DUP15q it is critical that these mice have high construct validity. Hence, the genes that are targeted in our mice should be very similar to the mutations as observed in the patients. In addition, to justify studying these animals as models of disease, the mice also need to show high face validity, meaning that the phenotypes must resemble phenotypes observed in patients. When we want to generate mice to test a specific scientific question (i.e. does gene X in the 15Q11-13 locus contribute to the disease), we should thoroughly investigate (by extensive literature studies and in vitro experiments) if that gene is indeed of high scientific interest and potentially of critical relevance to brain function to justify a new mouse model that specifically addresses that hypothesis

B. Molecular and electrophysiological analyses on tissues/primary cells (Appendix 2): Although we can now generate iPSC-derived neurons, these neurons do not show the same mature properties of neurons as can be obtained from mouse primary brain tissue. Also, electrophysiological measurements require fine-tuned synaptic connections that are not present in neurons grown in a dish, hence we need brain slices to measure the deficits of these neurons. These experiments will help us assess the role of UBE3A at the molecular and cellular level and help us address the questions for Aims 1-4. The primary

neuronal/astrocytic cultures will be used to test targets or interactors of UBE3A in knock-down or overexpression studies.

C. Mouse behavioural studies (Appendix 3): Only when molecular studies look promising and warrant further research to investigate how the manipulation affects behaviour (which is the final output of the brain), we will perform behavioural assays. We will most often use our well-established behavioural test battery [6], which is now used by many labs all over the world to assess behavioural deficits caused by loss of UBE3A (**Aim 3**). In case we have identified a putative UBE3A target (**Aim 1**) we may perform a proof of principle study, by crossing AS mice with a mutant of the identified target (reduced- or overexpression) to test the effect of such a double mutant. Behavioural testing is also needed to assess the effect of neighbouring genes in the 15q11-13 gene cluster (**Aim 2**) and to identify which brain regions are underlying the behavioural deficits caused by loss of UBE3A (**Aim 3**). Some of these experiments may require the injection of 5.1 lid1c to manipulate the expression of certain genes in our mutants (eg. putative UBE3A targets or specific genes in the 15q11-13 region).

3.4.2 Provide a justification for the strategy described above.

Appendix 1: Generation of mouse models: During the next 5 years, new information may become available from our own data, partners, meetings or the scientific literature. Based on the new knowledge, we may deem it necessary to generate or import a new mouse line to better model the disorder or allow us to get a better understanding of the function of proteins involved in AS or Dup15q.

Appendix 2: Molecular and electrophysiological analyses on tissues/primary cells: With the aim to understand the pathophysiology of AS, we would like to interfere with the expression of not only UBE3A but also of its interactors /targets. To achieve this, we plan to:

1. use "tool compounds" such as antisense oligos (ASOs), short hairpin RNAs (shRNAs), short interfering RNAs (siRNAs), short activating RNAs (saRNAs), microRNAs (miRNAs), DNAs or viruses to downregulate or overexpress the genes in question. Which of the aforementioned tool compounds will be used depends on the level of downregulation/overexpression we need. Since AS is a neurodevelopmental disorder, we would most likely carry out our intervention with tool compounds at an early age, such as in utero or directly after birth and administered using intracerebroventricular injections.
2. Setup breedings between our current mouse lines, or to achieve difference is gene dosages. As an example, the effect of levels of (neighbouring gene) interacting proteins such as HERC2 relative to UBE3A can be looked into by crossing our dup15q mouse line mice with our UBE3A overexpressing lines.

Appendix 3: Mouse behavioural studies: These experiments allow us to determine the face validity of our animal models, ie. do they display a phenotype and whether the phenotype(s) are stronger or milder. Also here, the administration of tool compounds may help us determine the role of UBE3A or (neighbouring gene) interactors.

In terms of animal wellbeing**, the strategy is designed in such a way as to:**

(a) keep the animal numbers as low as possible. When possible, we use **genetically engineered (immortal) cell lines or iPS cells for initial charachterization or for testing of tool compounds.** Another example to keep the animal numbers low is the isolation **and storage of tissue** after each experiment (when possible), and **to perform brief molecular studies to assess the validity of the line before deciding to do behavioural studies. And only when we observe behavioural deficits will we perform detailed molecular and electrophysiological studies.**

(b) Keep discomfort scores as low as possible. The initial work is first performed in vitro. This reduces the number of in vivo experiments. We will **first perform a limited molecular**

characterisation to study the effect of our (genetic) manipulation, to keep discomfort as low as possible. If needed, we then proceed to electrophysiological studies. Only when we have found molecular or electrophysiological changes that warrant behavioural studies to study the effect on brain function, we will **finally perform behavioural studies**

(c) Avoid the generation of new animal models when possible. We will only generate mouse models if they are not available elsewhere and if they address a major question in the field that is of large clinical or scientific value. As the generation and characterization of new models is very expensive, the final decision is ultimately decided by the reviewers of our grants. All our mouse models are made available to the field before or upon publication.

3.4.3 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	Generation and breeding of (new) GA mouse lines with possible discomfort
2	Collection of mouse tissue
3	Behavioural analysis of mice
4	
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Generation and breeding of (new) GA mouse lines with possible discomfort

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.

To understand the fundamental mechanisms underlying the pathophysiology of UBE3A related disorders, we make use of mice to perform ex-vivo and in vivo experiments. Preferentially, these studies will be carried out on mouse models that already exist. When, after a literature review and also consultations with our extensive international network of partners, a suitable mouse line is not found, we will review the need to make a mouse model ourselves. Important factors in such an evaluation are the potential scientific and/or clinical impact of the mouse model, whether or not we expect a harmful phenotype. We will then submit a grant proposal that is subjected to peer review (typically patient organization, ZonMw, EU). If awarded, we will proceed to design and generate a new genetically modified (GA) line.

De novo generation of new GA lines: The genetic modification is applied to embryos or embryonic stem cells, and GA animals are created by:1) injection of GA embryonic stem cells (ES) in blastocysts. 2) Injection of DNA/RNA constructs in oocytes. 3) Modification of oocytes by new techniques for gene editing, such as those employing CRISPR/Cas9. We anticipate to **generate a maximum of 2 new lines** in the next 5 years.

Breeding of a GA line with a mild harmful phenotype: Currently, none of our lines has a harmful phenotype. However, it is possible that we will generate or import GA mice with a mild harmful phenotype. We anticipate to **breed maximally 1 line with a mild harmful phenotype** in the next 5 years.

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

Table 1 Types of procedures and level of discomfort*)

	Gender	Goal	Procedures	Discomfort
1. Donor females	F	Embryo isolation	Mate with fertile stud / Plug check / Cull mouse by cervical dislocation / Harvest embryos, i.e. zygotes, morulae or blastocysts	Mild
		Oocytes isolation	Superovulation (2 i.p. injections, SOP) / Cull mouse by cervical dislocation / Harvest oocytes	Moderate
2. Fertile stud male	M	Fertilization	Mate with female donor mouse (max. 3x per week, 4 months active)	None
3. Vasectomized stud male*	M	Mock fertilization	Mate with foster mother (continued use)	Moderate (continued use)
4. Foster mother	F	Oocyte or embryo implantation	Mate with infertile stud male / Plug check next day / Surgical embryo implantation under injection anesthesia and pain relief (SOP) / Birth of GM pups / Wean litter / Cull mouse	Moderate
5. Founders	M & F	Identification DNA extraction and analysis	Distal phalanx clipping of mouse at 4-7 days after birth (SOP)) for identifying and genoptyping. In exceptional cases, an additional clipping of the ears, tail tip clipping or a blood sample is needed for analysis of the genetic alteration.	≤moderate<1%
6. Harmful phenotype	M & F	Breeding /maintaining	Animals are maintained /bred with a mild harmful phenotype	Mild

*) Vasectomized stud males will be used from the cryopreservation/rederivation program running in the facility

Four procedures involve treatment of the mice: 1) superovulation, 2) implantation 3) vasectomy and 4) distal phalanx clipping or clipping of the ear.

Superovulation (oocytes): Approximately 5-week-old female mice receive 2 i.p hormone treatments, 48h apart. Based on our experience, superovulation at this age results in a maximum number of fertilised oocytes of good quality for most laboratory mouse strains. Superovulation of older mice dramatically reduces the number of oocytes. After the last treatment, the female mouse is mated with a stud male. Next day a plug check is performed. Plugged females will be collected and set aside till usage. Plugged female mice are culled 0.5-3.5 days post mating and oocytes are isolated. SOPs apply.

At the 5.1 lid2h , the choice to not super-ovulate donors prior to blastocyst isolation is a conscious one and is based on the fact that in their experience the quality of blastocysts is much lower after superovulation when compared to natural mating. In the end in turns out that a lower number of blastocysts but of higher quality vs higher number of low-quality blastocysts equates to an equal number of mothers used with the added benefit that the discomfort is lower (mild vs moderate). Also, important to note is that with natural matings, only plugged mothers are used.

Implantation: Two types of implantations are performed. 1) oocyte/2-cell implantation in the oviduct of a foster mother. 2) blastocyst implantation in the uterus of a foster mother. Surgery is performed on 8-16 week old plugged female mouse (weight 18-30g) following SOP and takes on average 20 minutes per mouse. In brief, a 1 cm incision is made parallel to the dorsal midline to expose the oviduct and uterus.

For the oocyte/2 cell implantation: The infundibulum is located and using a fine glass capillary pipet 20-25 oocytes/2-cell embryos are inserted into the oviduct.

For the blastocyst implantation: With a syringe a small hole is made in the uterus wall. With a small glass capillary pipet 8-10 blastocysts are inserted through the hole into the uterus.

Next the oviduct-uterus is placed back in the abdomen and the peritoneum is closed with 1-2 sutures. Wound clamps are used to close the skin, which are removed 8- 10 days after the operation. Mice are anesthetised and post operation pain relief is administered.

We are aware of the non-surgical embryo transfer (NSET) procedure which potentially lowers the discomfort of blastocyst recipients. The procedure has been tested in our facility but results in a lower number of pups compared to surgically placing back the blastocysts. So, although the present procedure of surgical implantation of blastocysts does lead to an increased number of donors and foster mothers, ultimately it does reduce the number of repeated injection attempts.

Distal phalanx clipping: Distal phalanx clipping of mouse at 4-7 days after birth following SOPs for identifying and genotyping

Selection of the most suitable founder lines: Only those newly generated founder lines that have germline transmission AND an appropriate level of GA effect (normally expression of the GA gene in specific tissues/cell types) will be selected to produce offspring for procedures in the other appendixes. Assessment of an appropriate level of GA effect may include (trans)gene (in)activation by eg tamoxifen treatment, continuously or intermittently and via different routes (eg. in max 4 months in feed and/or water; max 5 x IP (5ul/g), or exceptionally - when other methods do not produce the desired result - once ICV (max. 5ul) and subsequent target tissue analyses.

Initial Welfare assessment: As described in the EU directive (2010/63/EU: corrigendum 24-01-2013), various parameters are checked daily. New mouse lines will be monitored for two generations to determine whether there is a harmful phenotype. In those mouse lines that have a harmful phenotype, the breeding of affected animals itself will be registered as (a part of) a procedure. Mouse lines with a more than mild harmful phenotype will not be included in this project.

Maintaining a colony of a GA line with mild harmful phenotype (maximum mild severity): We request one group of mice to allow breeding and maintaining one line with a mild harmful phenotype.

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

Statistical analyses are typically not being performed for these kinds of experiments, except for testing whether alleles transmit in a Mendelian fashion.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice GA	N/A (Annex I species specifically bred)	Pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129
	mice normal	N/A (Annex I species specifically bred)	adults		Both	No	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	Mus musculus: The body of knowledge on the mouse brain is large, and the development of the central nervous system exhibits a high degree of similarity to that of the human CNS, which makes the mouse a good model. Another important factor is the technology to make genetic changes, which allows us to use GA mice as models for UBE3A related disorders.
Origin	All animals are bred in the institute or come from a registered supplier and are housed under the same standard conditions. Animals will be socially housed whenever possible

Life stages	Mus musculus: genetically altered (GA) and non-GA animals of defined standard genetic backgrounds, both male and females, typically up to an age of 240 days. Both females and males must be sexually mature to be used in these experiments for them to act as donors (embryo/oocyte/sperm) or as foster mothers. Adult founders will be used for breeding the F1.
Number	Mus musculus: normal (non-GA) animals as well as GA mice of defined standard genetic backgrounds, both male and females, typically up to an age of 240 days. All animals are bred in house or come from a licensed commercial supplier. We estimate to generate a maximum of **2 new GA mouse lines** in the next 5 years. On average, 5.1 lid1c are required to generate a GA mouse line. 5.1 lid1c Initial characterisation of the founder lines (5.1 lid1c primarily involves selection on the basis of DNA sequence. After selection and breeding of the correct founder lines, further characterisation at both the genomic level as well as the protein level is necessary. In our experience with generating GA mice, more extensive characterisation of the inducible allele in the offspring from of each founder is necessary and how this affects protein levels needs to explored. This involves crosses with Cre- expressing lines to obtain tissues to test on Western blot, immunofluorescence labelling etc. 5.1 lid1c are humanely killed to collect tissue for a more extensive analysis to select the correct founder line, using for example Western blot, immunofluorescence or QPCR (to check for the correct change in protein levels/ DNA). **Maintaining a colony of a GA line with mild harmful phenotype:** All our current muse models, including both 5.1 lid1c **do not** show any harmful phenotype and therefore we anticipate that the newly generated lines or imported lines are likely to have no harmful phenotype as well. We can, of course, not guarantee this to be the case for the new lines to be generated or imported. In the worst-case scenario, 1 or our newly generated lines or imported lines will display a mild harmful phenotype. For this line we estimate that we will need about 5.1 lid1c for the 5-year period.
Gender	For the majority of experiments, mice of both genders can be used. There are exceptions such as egg donors and fosters being female and vasectomised males being male for obvious reasons.
Genetic alterations	For the generation of mouse models, it is important that the mutation encountered in patients is also introduced into the mouse genome to create a model with a high construct validity. This in turn increases its face validity. It is not possible to mention which mutations will be modelled here but will be identified in the study-plans. Initial welfare Assessment: We plan to generate and import new GA mouse lines during the next 5 years. In line with the recently revised guideline for genetically modified animals, a welfare assessment will be carried out to monitor and determine the welfare of new transgenic animals (2 generations, 7 males and 7 female control and GA mice). These animals will most likely be added to a new general project license. If that license is not in place in time, we will write an addendum.
Strain	We will use normal (non-GA), genetically altered (GA) and wild-type control mice in different standard genetic backgrounds (eg Bl6, 129 and FVB) or combinations (eg F1 Bl6 and 129) thereof depending on how the genetic background affects the phenotypes we see in the strains. The strains to be used will be mentioned in future study-plans.

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

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

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

Surgical procedures, including embryo transfer and vasectomy, will be carried out under general anaesthesia with adequate peri and postoperative analgesia.

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

In many cases it is difficult to predict exactly what the effect will be of an alteration of the DNA sequence of the mouse genome. From our experience, none of our AS mouse models 5.1 lid2h) show any deleterious effect on their well-being, and phenotypic changes we measure are only observed through advanced experimental testing where specific phenotypic modifications can be detected. However, since we may also generate models involving genes other than Ube3a, in 40% of the newly generated GA lines we can potentially expect a mild harmful phenotype such as susceptibility to epilepsy. It is difficult to predict beforehand the severity of the phenotype we expect as this can differ between humans and mice. When mouse lines do experience an intrinsic discomfort severity beyond mild, the line will no longer be used and the generation of an inducible line will be considered.

Explain why these effects may emerge.

These effects may emerge because of the role of the gene in brain development and their resemblance with AS/ Dup15q patient (of which most have epilepsy).

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

If unexpected adverse effects are observed, immediate action will be taken. This could be through removal of a specific animal from the experiment, or even termination of the entire GA mouse line in favour of a more refined model (e.g. inducible mutant).

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

All mice will be observed daily with respect to several parameters (overall appearance, size, confirmation and growth, coat condition, behaviour, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013.

Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:

1. Stunted growth (>15% reduced weight compared to littermates).
2. Moderate circulatory or respiratory problems.
3. The development of a clinical neurological disease that results in sustained suffering **(**eg they display deviant behaviour such as abnormal aggression, ataxia, lethargy or epilepsy**)**.

For animals that underwent surgery, we will also humanely kill the animal if two days after the start of the experiment the animal has a decrease in body weight of maximally 20%, with a recovery within 2 days post-surgery to a max of 10% (relative to their weight at the start of the experiment).

Indicate the likely incidence.

Less than 1%: We have extensive experience with generating new GA lines in the last 20 years, which include all our current Ube3a related models including both the large deletion AS model as well as the large duplication (Dup15q) mouse model. So far, none of these lines show any harmful phenotype and very rarely do we have to kill an animal because the above criteria are met. Since breeding of animals with abnormal behaviour/appearance increases the likelihood of affected offspring, animals are typically killed long before the humane endpoint criteria are met.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Donors: multiple injection of hormones to induce super-ovulation. Humanely sacrificed and eggs harvested (severity: Mild)

Fosters: These mice will undergo abdominal surgery to place back the blastocysts or injected eggs. Humanely sacrificed after weaning (Moderate) Vasectomised males: Males undergo abdominal surgery for sterilisation purposes (Moderate)

Offspring and breeding: These mice do not undergo any treatment. There is a potential for breeding with a mild harmful phenotype but that is difficult to predict (Severity: mild). The emergence of a mild harmful phenotype depends on the protein involved, and can cause for example, mothers to be more prone to stress and becoming more aggressive. As a consequence, some of the pups may be cannibalised by the mother within the first 14 days after birth. Mutations introduced into some mouse lines may result in an increased seizure susceptibility the frequency of which is at most 5/day This is determined during the extensive analysis of each line when mice will be observed with cameras and footage analysed to detect the presence of seizures. When the severity of the experienced harmful phenotype is deemed too high, the mouse line will no longer be used and the generation of an inducible mouse model may be considered (Severity: Mild).

All mice are humanely killed.

Mice normal

Severity	#	%	Treatment Groups
3 Moderate	160	80%	Female donors
3 Moderate	30	15%	Foster moms
2 Mild	10	5%	Vasectomized stud males (will be used from cryopreservation/rederivation program, hence not included in total)

Mice GA

Severity	#	%	Treatment Groups
2 Mild	100	14%	Founders
2 Mild	100	14%	tissue collection for analysis F1
2 Mild	500	71%	Maintenance of GA line with a mild phenotype

G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to modify the gene and to study the effect of the genetic modification on brain development and brain function. To date, studying GA mice is the only reliable method to investigate the function of a gene in brain development and brain function *in vivo*. Before we embark on choosing the genes for generating novel GA lines, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation.
Reduction	The vasectomised males used in this study also participate in the cryopreservation/rederivation program that is ongoing in the transgenic core facility, thereby obviating the need to use a separate cohort of mice.
Refinement	The lab has obtained extensive experience in the last 2 decades in the type of procedures been described and knows how to determine when mice are approaching humane end points such as weight loss, trembling, fur condition etc. The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of neurodevelopmental disorders, and participate in scientific meetings. Also, we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study. We continuously strive to reduce the discomfort the mice are subjected to, and although some labs do use the NSET procedure, a non-surgical procedure to place blastocysts back into the recipient, experience with this procedure in our facility resulted in a lower number of pups compared to when we used the surgical procedure, described in this application. We do recognise that the present procedure of surgical implantation of blastocysts does involve a higher discomfort to the mice, we believe that the higher number of mice/injection obtained in our hands does ultimately reduce the number of repeated injection attempts.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

A number of the animals will be humanely killed after general anesthesia, eg. egg donors and foster mothers (at weaning) in the course of the experiment. Non-GA offspring will be killed before weaning.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☒ No > Describe the method of killing.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia. This causes least pain andstress for the animals

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons, as part of the procedures or to resolve welfare concerns.

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1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 2 — Type of animal procedure: Collection of mouse tissue

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.

To address the research questions of this application, we will make use of *ex-vivo* experiments as much as possible. This appendix describes the collection of

1) Embryonic brain tissue to setup primary neuronal cultures will be used to determine the role of UBE3A protein in the different cellular compartments (**aim 1**) and to determine the effect of AS mutations or the role of neighbouring genes (**aim 2**) on selected readouts.

2) These same questions will also be investigated at different ages in vivo, by collecting brain tissue and subjecting these to various biochemical and electrophysiological analyses (**aims 1-2**). In addition, brain tissue is also required for molecular or electrophysiological studies to study the role of UBE3A in specific brain regions (**aim 3**) To address these aims, we will use normal and GA (single, double or triple) mice. In order to obtain the tissues, animals are humanely killed after general anaesthesia, and tissues are dissected for further analyses using biochemical/molecular, electrophysiological and imaging methods (microscopy analyses).

For **all aims,** we may administer Tamoxifen to induce expression of Floxed alleles. We may also administer 5.1 lid1c to the animals before we collect the tissue.

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

Tissue collection of >P21 mice. Mice will be humanely killed after general anaesthesia.

Tissue collection of newborn animals: In case we need to collect tissue from newborn mice for subsequent analysis, pups are rapidly cooled on ice water to provide hypothermia-anaesthesia, and decapitated to obtain the brain for further analyses.

Administration of tool compounds to modulate gene expressions: Prior to tissue collection, animals may be subjected to **maximally 5 IP injections of tamoxifen** (maximum volume of 5ul/g) to induce brain- specific gene deletion/activation in the case of floxed alleles.

Animals may also be subjected to the administration of the molecules to modulate specific gene expression levels. These 5.1 lid1c

Administration via ICV in neonates: this involves subjecting the neonates to hypothermia-anaesthesia using iced water and injecting the compound directly into the lateral ventricles. The whole procedure takes about 5 minutes after which the pups are placed under a heating lamp to recuperate.

In older mice, administration of tool compounds involves placing the mouse under general anesthesia with analgesia, moving the skin to allow the drilling of a small hole in the skull using the stereotactic device followed by suturing of the skin and wound care. The whole procedure takes approximately 30-40 min.

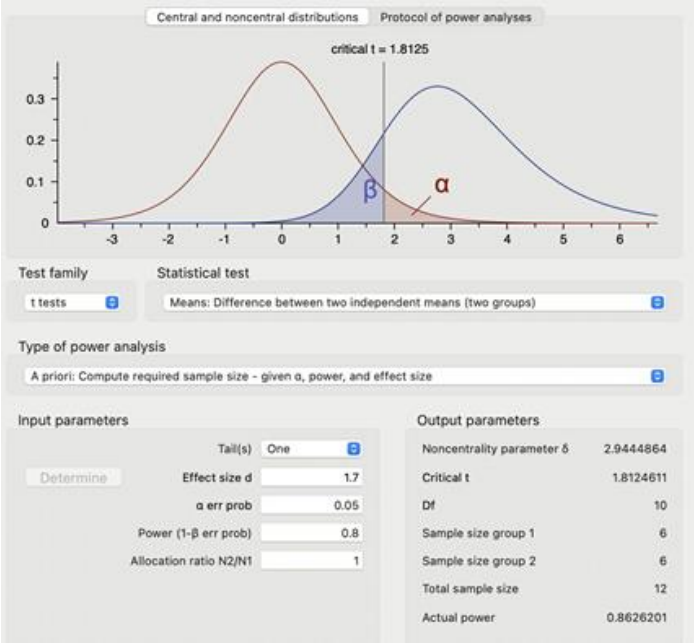
It is important to note that the cumulative discomfort endured by each animal due to the administration of these compounds will never exceed moderate discomfort.

For imaging analysis, some of the above mice will be sacrificed and subjected to transcardial perfusion after general anaesthesia (using an overdose of Nembutal (Pentobarbital)).

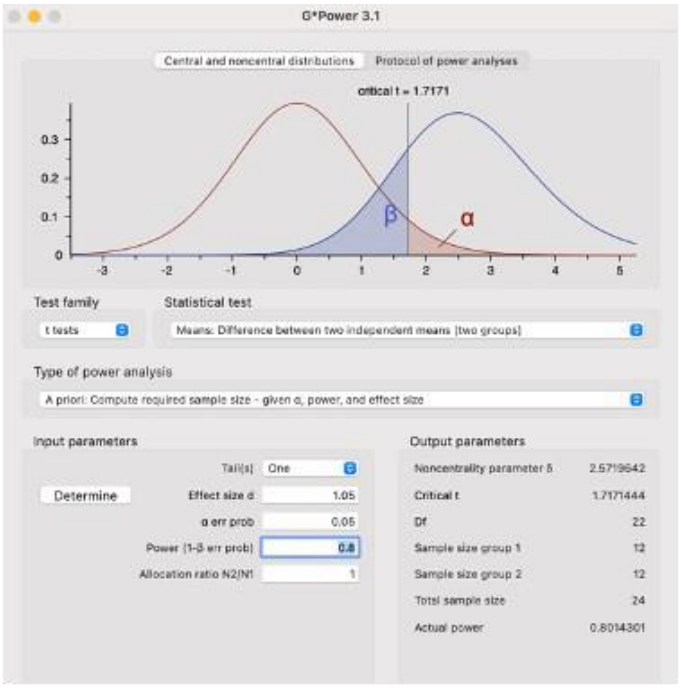
Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We use tissue for a large variety of experiments, each requiring different analyses and sample sizes. In most cases the number of animals will be based on literature and/or (more likely) on our previous experience with similar experiments. The experiments are often of explorative nature and will be carried out using a minimum number of mice per group to detect meaningful effect sizes (β of .8 and an alpha (α) of 0.05). The numbers are mostly based on previous experience and strongly depend on the outcome measure. The experimental design of each experiment including the numbers of animals and type of analysis will be submitted to the IvD.
Through careful randomization we try to minimize the number of animals by reducing the effects of potential differences in age, sex and litters. For our statistical analysis we do not take such possible differences into account, which, for this type of explorative experiments is the standard in the field.

As an example, for molecular Western blot analyses in which we want to be able to detect a difference of 20% of the means, we typically need 6 mice/group:



As an example for electrophysiological analyses in which we want to be able to detect a difference of 20% of the means, we typically need 12 animals/group:



B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice GA	N/A (Annex I species specifically bred)	Pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129
	mice normal	N/A (Annex I species specifically	Pups and adults		Both	No	eg. Bl6, FvB and 129

		bred)					

Provide justifications for these choices	
Species	*Mus musculus*: Knowledge about the mouse brain is large, and the development of the central nervous system exhibits a high degree of similarity to that of the human CNS, which makes the mouse a good model. Another important factor is the technology to make genetic changes, which allows us to use GA mice as models for neurodevelopmental disorders.
Origin	All animals are bred in the institute or come from a registered supplier and are socially housed, whenever possible, under the same standard conditions. This ensures the animals are genetically pure and free of known infectious agents.
Life stages	Mice at all stages (from E14.5, neonates, juvenile, adolescent or adult) will be subject to biochemical, immunohistochemical, ePhys analyses after treatment with therapeutic/vehicle. This allows us to properly study the different developmental stages of the mice. The chosen age will depend on the functional test and whether the age is suitable for the test. The treatments described for the induction of the GA alleles and/or treatments with drugs will in some cases begin before parturition, to study the effects on the embryos during development.
Number	The numbers detailed below are a maximum we will expect to use. The actual numbers and conditions will be outlined and justified in the study-plans presented to the IvD for consent prior to performing any experiment. In the proposed experiments we will make use of a maximum of 6 GA mouse lines. Below we delineate the number of mice needed for each type of experiment. Typical group sizes: To address a research question using neuronal cultures, we typically use 5 independent cultures (5 dams, with on average 4 foetuses each (6 for normal, 4 for GA animals). To perform a detailed biochemical (e.g. proteomics, Western blots, kinase assays), genetic (e.g. gene expression (RNA-seq) analysis) and imaging analysis (e.g. immunofluorescence) we typically need 6 animals per group. For slice electrophysiology we typically record a number of different electrophysiological parameters from different cell-types, different brain areas, and different developmental ages. From previous experience we know that the recording of a single measure typically requires 12 animals per group. **I. Animals for neuronal cultures (**5.1 lid1c **)** In Aims 1 and 2 we look into how specific mutations lead to a change in localisation and how UBE3A interacts with its "targets" in addition to deciphering the role of UBE3A in the nucleus/cytosol/synaptic compartments. We estimate that over 5 years we will perform 20 studies involving GA animals and 20 studies involving normal animals. With a group size of 5 dams per study, this requires: 5.1 lid1c The dams will be sacrificed and embryo's extracted to setup the neuronal cultures. **Total: 200 dams + 1000 embryos = 1200 animals** **II. Animals for tissue collection without additional procedures or discomfort (**5.1 lid1c To charachterise our recently developed mouse models and future mouse models we will perform electophysiological analysis and molecular analysis on mice with different genotypes. Depending on the used genotypes, most experiments have 3 groups (WT, HET, HOM), 4 groups (WT, Het; plus or minus Cre) or up to 8 subgroups when double

	mutants are used. We expect that in the coming 5 years we 5.1 lid1c We expect that in the coming 5 years 5.1 lid1c **III. Animals for tissue collection with a mild harmful phenotype** 5.1 lid1c Although currently none of our animals has a harmful phenotype, it is possible that interbreedings of current mutants or generation/import of novel GA lines yield animals with a mild harmful phenotype. We expect that in the coming 5 years we will 5.1 lid1c We expect that in the coming 5 years we will 5.1 lid1c **IV. Animals for tissue collection after IP injection** 5.1 lid1c Many of our mouse models are inducible, meaning that upon tamoxifen injection we can delete a gene ('Floxed' genes) or reinstate gene expression ('Floxed-Stop'). This requires 5 IP injections with Tamoxifen. A typical experiment with inducible alleles has 4 groups (Mutant, Wildtype with and without CRE, all receiving Tamoxifen). We expect that in the coming 5 years we will 5.1 lid1c . We expect that in the coming 5 years we will 5.1 lid1c **V. Animals for tissue collection after ICV injection with tool compound** 5.1 lid1c 5.1 lid1c . Such experiments typically has 4 groups (Mutant, Wildtype with and without tool compound). 5.1 lid1c We expect that in the coming 5 years we will submit 5 5.1 lid1c We expect that in the coming 5 years we will submit 5.1 lid1c
Gender	Most phenotypes do not differ between male and female patients and mice, and hence we will use both sexes. In the event there is a gender bias, we will choose the gender with the strongest phenotype.
Genetic alterations	For our research, it is vital that we use mouse models that carry mutations similar to those encountered in patients giving the models a high construct validity which in turn translates to a high face validity.

Strain	We will use normal (non-GA) control and genetically altered (GA) mice in different standard genetic backgrounds (eg Bl6, 129 and FVB) or combinations (eg F1 Bl6 and 129) thereof depending on how the genetic background affects the phenotypes we see in the strains.

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

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

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

The discomfort depends on how the tool compounds are administered. This can be through IP, SC or IV injections without the use of anaesthesia. When mice are injected ICV or intracerebrally, hypothermia-anaesthesia (P0-P3) is applied. For implantation of an osmotic pump (1x) or administration via intrathecal or intracerebroventriuclar (ICV) injection or via a cannula, the procedure is carried out only once/mouse and requires surgery with adequate general anaesthesia and perioperative analgesia.

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

Tool compounds can potentially have unforeseen side effects. Also, it is possible that the animals do not recover well from surgery after ICV injections. Also, complications such as infections at the site of injection may occur.
From our experience, most genetic modifications themselves have no effect on the well-being of the animal, and phenotypic changes are only observed through advanced experimental testing where specific phenotypic modifications can be detected. Most GA animals will not have a harmful phenotype. However, we can potentially expect a mild harmful phenotype such as epilepsy when we cross two mutants, and this has been accounted for in our calculations. It is difficult to predict beforehand the severity of the phenotype we expect as this can differ between humans and mice. In some cases where harmful phenotypes are expected, the generation of inducible models may be the solution.

Explain why these effects may emerge.

Some of the new lines have not been characterised and although the impact of most procedures on animal welfare are well known, the effect of the tool compounds are unknown and may elicit side effects.

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

If unexpected adverse effects are observed, immediate action will be taken. E.g., the drug dose will be reduced or treatment may be withdrawn altogether. If appropriate we will administer pain medication or remove the animal from the experiment and euthanise it.

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

All mice will be observed daily with respect to several parameters (overall appearance, size, confirmation and growth, coat condition, behaviour, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013

Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:

1. Stunted growth (>15% reduced weight compared to littermates).
2. Moderate circulatory or respiratory problems.
3. The development of a clinical neurological disease that results in sustained suffering (eg they display deviant behaviour such as abnormal aggression, ataxia, lethargy or epilepsy).

For animals that underwent IP or ICV injection of tool compounds, we will also humanely kill the animal if two days after the start of the experiment there is a decrease in body weight of maximally 20%, with a recovery within 2 days post-surgery to a max of 10% (relative to their weight at the start of the experiment, or for juvenile mice, compared to untreated littermates), or if mice experience any discomfort during the entire experiment such that the cumulative discomfort would rise beyond "mild".

Indicate the likely incidence.

Less than 1%: We have extensive experience with generating new GA lines in the last 20 years, which include all our current Ube3a related models including both the large deletion AS model as well as the large duplication (Dup15q) mouse model. So far, none of these lines show any harmful phenotype and vary rarely do we have to kill an animal because of the above criteria. Since including an individual animal with even mild discomfort can affect the measurement outcome, animals are likely to be killed well before the above criteria are met.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Figure 2: Summary of estimated discomfort scores.

For neuronal cultures or for the isolation of brain tissue, mice (or embryos) are sacrificed humanely without any prior procedures (Severity: Mild).

For some new lines or crosses, a mild harmful phenotype may be present during their life span prior to sacrificing (Severity: Mild)

To manipulate the expression of inducible alleles, mice may receive (max 5) IP Tamoxifen injections prior to sacrificing (Severity: Mild).

To manipulate the expression of other target genes, mice may receive a single ICV injection with tool compound under anaesthesia prior to sacrificing (Severity: Moderate).

G. 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	Most experiments are performed using cell culture that requires no animals. When we use tool compounds to manipulate gene expression, they are first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when a tool compound is found to function well in in vitro and ex-vivo experiments, will it be tested in vivo, which reduces the number of animals we use.
Reduction	Before we embark on choosing the genes for generating novel GA lines, we pre-screen mutations in cell lines in addition to using neuronal cultures at a later stage to select the most appropriate mutation. We also employ some mice for *in utero* manipulation of the brain to determine whether this causes a phenotype. Once we decide to test a therapeutic, this is first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when the therapeutic is found to function well in in vitro and ex-vivo experiments, will it be tested in vivo, which reduces the number of animals we use.
Refinement	The lab has obtained extensive experience in the last 2 decades in the type of procedures been described and knows how to determine when mice are approaching humane end points such as weight loss, trembling, fur condition etc. The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of neurodevelopmental disorders, and participate in scientific meetings. Also we check through extensive literature searches (Medline, Current

	Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The procedures of this appendix are aimed at obtaining brain tissue, which requires killing the animal.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.

Mice will be sacrificed to obtain the tissues used for subsequent analyses. Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons or to resolve welfare concerns.

Centrale Commissie Dierproeven

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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 3 — Type of animal procedure: Behavioural analysis of mice.

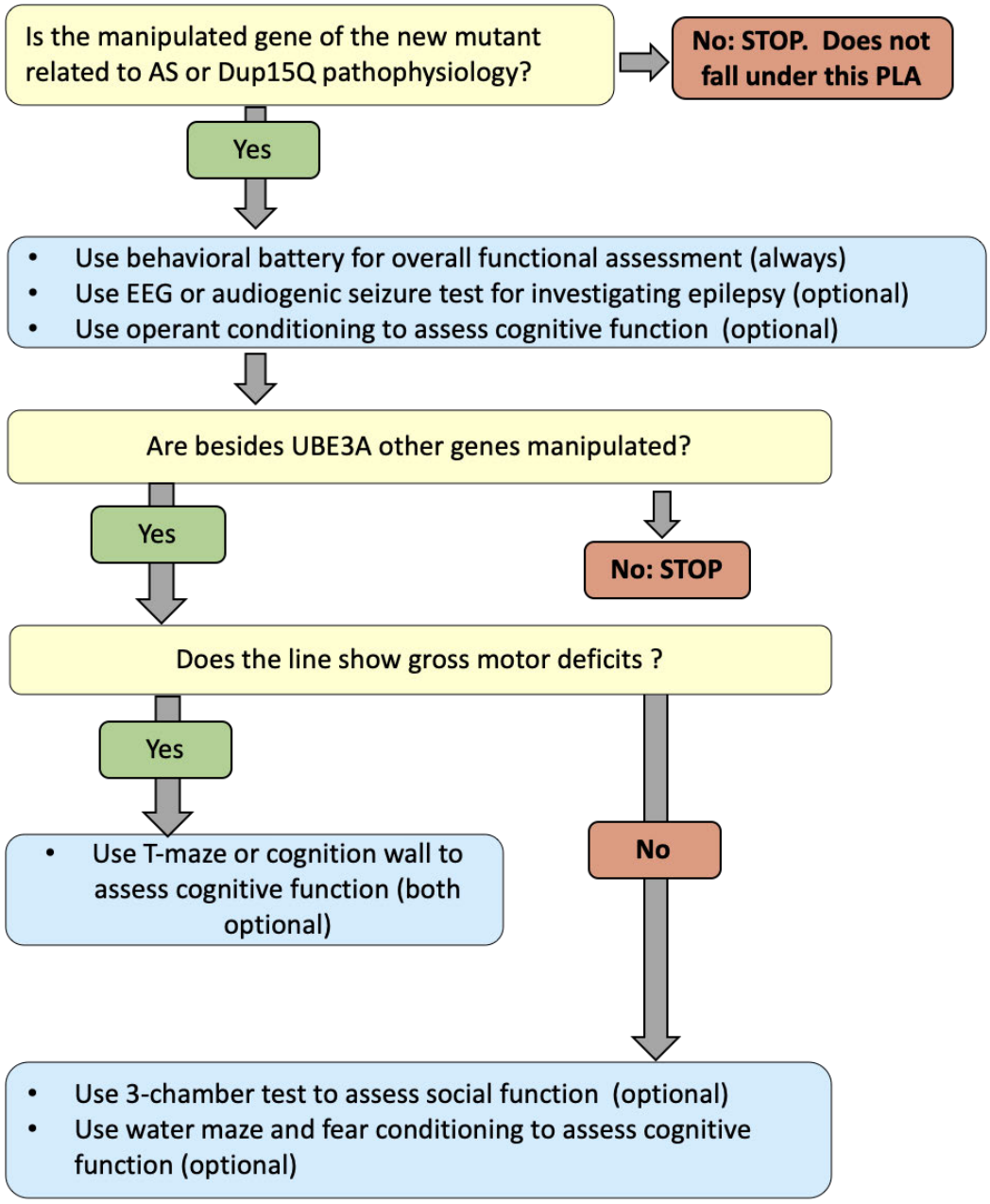
2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

Behavioral change is the ultimate read-out of our brain. Mouse models for neurodevelopmental disorders often recapitulate the main features: **intellectual disability, abnormal behaviour, motor deficits, sleep deficits, abnormal EEG and epilepsy**. This research concerns the behavioural analyses performed on existing or newly generated (**Appendix 1**) AS/Dup15q mouse models using behavioural tests. This appendix includes the standardized behavioural test battery that was optimized and implemented in the lab to efficiently and objectively assess phenotypes as observed in AS mice. It also includes additional tests that may yield a phenotypic difference between WT and mutant littermates. New and existing mouse models will be subjected to these tests to establish whether or not phenotypic differences are observed and can be used as a functional assay to determine the effect of genetic, therapeutic or pharmacological intervention in the future. We also want to test additional paradigms to be able to detect novel phenotypic differences between GA and WT mice. These experiments are needed to address **aim 1** (role of cellular UBE3A in brain development), **aim 2** (synergy between UBE3A and neighbouring genes) and **aim 3** (identify brain regions affected by loss of UBE3A).

For **all aims,** we may administer Tamoxifen to induce expression of Floxed alleles. 5.1 lid1c



Strategy to characterize the new mouse lines

The rational to use each test is explained below. Note that the UBE3A-sensitive tests (behavioral battery, seizures and operant condition) are routinely used in our lab and other labs in the world to test UBE3A-related deficits.

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

A). A highly standardized behavioral test battery for gross motor performance and innate behaviours, for which we have shown that it is sensitive to the loss of UBE3A. This tests battery is now widely used in the AS field. Mice that solely have a UBE3A manipulation will only be run in this test battery.

We have set up a behavioural test battery which probes motor issues, and a number of innate behaviours such as anxiety issues and behaviours that are often affected in AS mouse models. For the tests described below, a single cohort of (treated) mice, comprising of mutant and WT control mice, will be subjected to a maximum of 7 tasks for which we have demonstrated a difference in performance between WT and mutant mice. After extensive handling of the mice to decrease stress and to get the animals acquainted with the experimenters, all mice in the cohort are sequentially subjected to these tasks over a period of 2-4

weeks. The most stressful task (5.1 lid1c (highest discomfort score) is done last, so as to not interfere with the outcome of the earlier tests. After the behavioural testing, the mice are humanely killed to analyse the brain tissue. The animals are carefully acquainted to handling before they testing ensues to minimize stress levels. The animals are randomly selected to be tested and the handler/observer (tester) is blinded for the specific genotype or treatment. With the exception of the 5.1 lid1c , all tests are considered mild discomfort, but the sum of these tests result in a cumulative discomfort of moderate.

The battery has the following tests and is performed in this order:

Hind limb clasping reflex (max 10s, twice)(Severity: mild). Hind limb clasping is a marker of motor impairment and cerebellar dysfunction. The clasping test evaluates the animal's hind limb response during tail suspension 10 cm above the home cage. If the hind limbs are splayed out- ward, away from the abdomen, the mouse is assigned a score of 0. If one hind limb is retracted toward the abdomen, the animal receives a score of 1. If both hind limbs are partially retracted towards the abdomen, it receives a score of 2. The animal receives a score of 3 if the animal's hind limbs are entirely retracted and touching the abdomen.

Wire hang test (max. 60s once) (Severity: mild). The Wire hang test is used to measure subacute muscle function and fatigue. A horizontal wire (2 mm in diameter, 40 cm in length) is suspended 20 cm above a padded table. The animal is positioned to cling in the middle of the wire with its forepaws for one 60 s trial, and latency to fall is recorded.

Accelerating Rotarod or Accelerating Reverse Rotarod (2 trials of max. 5 min a day, for max. 5 days) (Severity: mild). This test measures motor coordination/performance. Mice are tested 2 times a day (ITI 1hour) on the Rotarod (a turning accelerating cylinder with a diameter of 3 cm (accelerated 4-40 rpm). This is repeated over a total of 5 days. The outcome parameter is the amount of time the mice can stay on the rod without falling. Maximum time per trial is 5 min. The reverse rotarod is the same as the accelerating Rotarod with the exception that the mice are placed on the rotating drum in the opposite direction, forcing them to walk backwards. This is a more demanding task than the regular accelerating Rotarod, and depending on the severity of the motor impairments we choose either one of these rotarod paradigms.

Open field (max. 10 min, once) (Severity: mild). This test measures anxiety and general activity. Mice are placed individually in a brightly lit 120 cm diameter circular open field for a period of 10 minutes and their exploratory behaviour is recorded on video and analysed by tracking software.

Marble burying (max. 30 min once) (Severity: mild). This test measures anxiety/repetitive behaviour. Mice are placed for 30 min. in a clean polycarbonate cage with a rich amount of bedding material. On top of the bedding material 20 blue glass marbles are arranged in an equidistant 5 x 4 grid and the animals are given access to the marbles for 30 minutes. Most (WT) mice will try to bury the marbles. The outcome is the number of marbles buried (visually inspected).

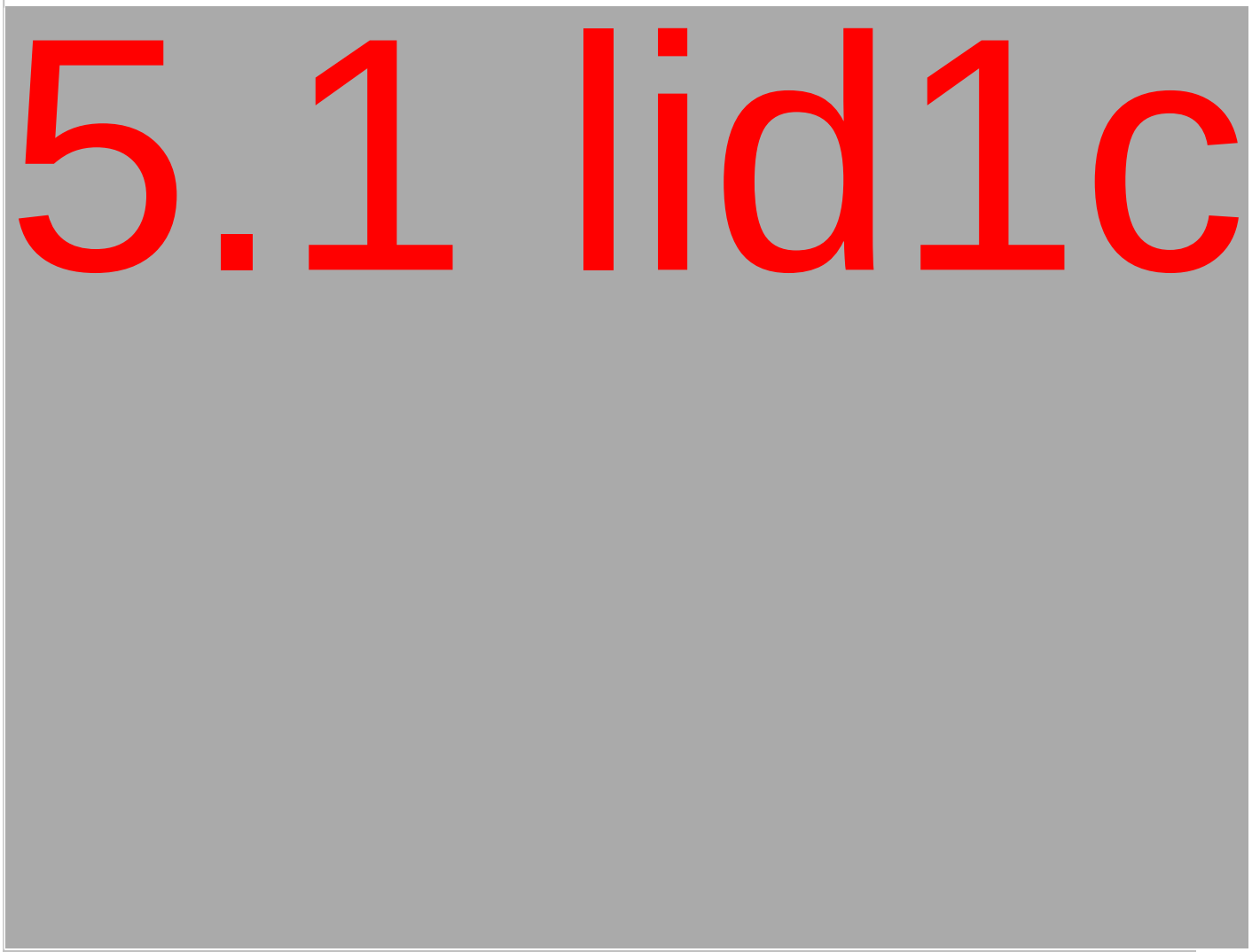
Nest building (max. 7 days continuous) (Severity: mild). This test examines the nest building behaviour of a singly housed mouse over a period of maximally 7 days. On day 1 of the test 3 cotton squares (nestlets) are added to the cage to be used by the mouse to build a nest. The weight of the remaining unused nestlets is determined each morning for maximally 7 days, allowing for the quantification of the nest building behaviour.

For novel mouse models, we may extend the characterisation with the following tests (each test requires a distinct cohort of animals and all tests are classified as moderate):

B. Cognitive tests:
These tests are very important to study whether deletion or duplication of other genes in the locus affects cognitive function. All tests described below require a **separate cohort** of mice. All these tests are classified as moderate.

Morris Water maze (max. 2 times daily for 1 min; for 14 days) The water maze test is a very sensitive test to probe spatial learning deficits as a measure of hippocampal function. As such, it is generally considered the gold standard.. In order to reduce the stress levels, a week prior to the start mice will be handled every day for 2-5 minutes. Handling consists of picking up the mouse and putting it on the hand or sleeve. Adaptation will show by more relaxed behaviour and some exploratory initiatives. This reduces the stress levels of the animals during the water maze test and consequently reduces the number of mice that have to be excluded from this test because of underperformance. During training, the mice will receive 2 trials a day for 60 sec to find the hidden platform just beneath the surface of a pool (diameter 1.20 meters) filled with visually opaque warm water (temp 25-26 degrees Celsius). Visual cues, such as coloured shapes or patterns, are placed around the pool in plain sight of the animal. After 1 min of swimming has passed the mouse will be placed on the platform for 30 sec. This will be repeated 5-14 days depending on the learning curve (finding the platform). At the end of the training, the mice will get a probe trial in which the platform is removed to see if the mouse has learned the location of the platform. Various parameters are measured including average distance to platform, latency to escape, path length, and velocity.

T-maze (max. 10 times 1 min; for 7 days): A T-maze is a simple maze used in animal cognition experiments. It is shaped like the letter T (or Y), providing the mouse with a straightforward choice. T-mazes are used to study rodent learning and flexibility when the task is modified. The animals are briefly (overnight) food deprived prior to the test and learn the test through positive reinforcement (food rewards). They can enter and leave both arms and the learning outcome is the immediate proper choice between the arms.



5.1 lid1c

C. Tests designed to study social deficits:

These tests are very important to study the role of a gene/protein in autism. If the mutation in question is linked in humans to autism, GA mice carrying the mutation may be subjected to these tests in an effort to determine the effect of treatment in reversing autism phenotype. All tests described below require a **separate cohort** of mice.

Three chamber test: (max. 1 hour; daily for 5 days). This test measures how much a test mouse interacts with a stimulus mouse (known or unknown) as compared to interaction with an object. The test mouse is placed in the centre compartment (43x40 cm) of the three-chamber set-up, and is monitored to explore the outer compartments containing either an object or a confined stimulus mouse.

D. Tests measuring brain activity and epilepsy: Also for both 5.1 lid1c **and audiogenic seizure tests, a separate cohort of mice are needed**

These test are important to study epilepsy, which is a serious clinical complication for Angelman syndrome and Dup15q syndrome. We consider these tests as moderate discomfort.

5.1 lid1c

Sensitivity to Audiogenic seizures (Max 20s twice): To determine the susceptibility of mice to audiogenic seizures, both mutant and WT littermates are subjected to the noise made by vigorously scraping scissors across the metal grating of a cage lid, which generates a 100dB noise. This is done for 20 seconds, or less if a tonic-clonic seizure develops before that time. A typical seizure lasts 5-15s, and the animals appear fully recovered within 30 seconds. This test is done at the start of an experiment (baseline) and if needed repeated one more time after a specific treatment.

E. Introduction of novel testing paradigms

It is possible that during these 5 years we may introduce a test that is not described here for testing AS mice. In particular in the domain of social tests (autism) and cognitive tests (learning disability) there is a need for better (more robust, less intense) tests. Such test will not exceed the discomfort of the tests described here, and will only be considered if it provides a clear scientific advance or is better with respect to reduction or refinement. For instance, we are interested in exploring the value of video based recordings to determine seizure frequency, which would reduce or eliminate the need of invasive EEG recordings.

Administration of tamoxifen or tool compounds to modulate gene expressions

Prior to behavior, animals may be subjected to drugs that activate/inactivate the gene/allele (induction). For example, the animals may receive maximally 5 IP injections of tamoxifen (maximum volume of 5ul/g) to induce brain-specific gene deletion/activation in the case of floxed alleles. Animals may also be subjected to administration of the 5.1 lid1c It is important to note that the cumulative discomfort endured by each animal due to the

administration of these compounds will never exceed moderate discomfort.

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

The minimal group size needed to obtain a significant difference in a particular behavioural test depends on the mutant mouse line in question. As an example, below is an example of the group size needed for Angelman syndrome mice. From extensive experience gathered over the past years, we have determined that groups consisting of 15 mice per genotype are sufficient to show a robust difference between controls and Angelman syndrome mice (7). Using a meta-analysis (7) the statistical power of the subtests was determined. In table 2 (see below) is indicated how many mice are needed for each behavioural test. In our study a power of 0.80 will be used, considering some unexpected variation we will use n=15. This provides us with sufficient statistical power to run a 1- or 2-way ANOVA and detect meaningful effect sizes. We typically do not consider phenotypes to be robust if more than 15 animals are required. On the other hand, we do not go below 10 animals per group because it limits our statistical power and analysis, and becomes too vulnerable for outliers. Experience has also helped us determine the number of mice need to run a well powered EEG experiment. We need 12 mice per genotype to detect meaningful effect sizes for each dose of therapeutic.For each new mutant mouse model, the tasks that will be employed will be based on what is known in the literature or expected, based on the nature of the mutation and gene involved. The presence of a performance difference between WT and mutant mice will first need to be verified for each individual task, and the robustness of the performance difference will determine the group size.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
	mice GA	N/A (Annex I species specifically bred)	Pups and adults	5.1 lid1c	Both	Yes	eg. Bl6, FvB and 129

Provide justifications for these choices

Species	Mus musculus: genetically modified and wild type littermates in different standard genetic backgrounds (eg Bl6, 129) or combinations (eg F1 Bl6 and 129) thereof. We choose this species based on the fact that they are genetically similar to humans and because we are able to test relevant parameters (eg. motor learning, cognitive tests, epilepsy etc) in mice that are often translatable to humans

Origin	All animals are bred in the institute or come from a registered supplier ensuring the animals are genetically pure and free of known infectious agents. The mice are socially housed, whenever possible, under the same standard conditions unless the behavioural test does not allow for this (eg. Nest building test). In those situations, the mice are single-housed for as short a period as possible.
Life stages	All behavioral tests are performed on adult mice. However, when behavior is combined with ICV injection of tool compounds, P1 or >P21 pups may be used for ICV injection.
Number	As explained earlier in the "experimental approach", from extensive experience gathered over the past 15 years, we have determined that for 5.1 lid1c . This provides us with sufficient statistical power to run a 1 or 2-way ANOVA and detect meaningful effect sizes. Smaller effect sizes can be detected by larger numbers, but the biological relevance of such small effects is often questioned. Conversely, given the variability of behavioural testing, smaller group sizes can readily result in Type I or Type II errors. For **audiogenic seizures and** 5.1 lid1c we use a group size of 5.1 lid1c **The number of groups** within an experiment is dependent on the complexity of the genotypes. For Cre lines as well ICV injection we typically have 4 5.1 lid1c Although double mutants may yield 5.1 lid1c . A larger group size would inflate the statistical power and is practically not manageable. 5.1 lid1c Below we estimate the maximum number of mice we will use in the coming 5 years. For each experiment the number of mice will be detailed and justified in the study plan. **I. Behavioral analysis without additional procedures or discomfort (5.1 lid1c Moderate)** To characterize our recently developed mouse models and future mouse models we will perform behavioural analysis on mice with different genotypes to perform one of the following: 1. The behavioural test-battery (single cohort) 2. Tests to measure Cognitive deficits (one cohort per test) 3. Tests to measure Social deficits (one cohort per test) 4. Seizure tests (one cohort per test) 5. Novel tests (one cohort per test) We expect that in the coming 5 years we will 5.1 lid1c We expect that in the coming 5 years we will submit max. 5.1 lid1c **II. Behavioral analysis with mild discomfort (5.1 lid1c ; Moderate)** It is conceivable that one of our lines shows mild discomfort or that mild discomfort arises when mutants are intercrossed. We are in particular alert for epilepsy, as we see this in both AS as well as Dup15 patients. For such mice, good quantification of seizures is needed. If the mice show motor deficits they can't be used for most of our tests, but we still need testing to assess the severity of the motor deficits. We expect that in the coming 5 years we will submit max. 5.1 lid1c

	We expect that in the coming 5 years we will submit max. 4 5.1 lid1c **III. Behavioral analysis upon IP injection (5.1 lid1c ; Moderate)** For our inducible lines we will use IP injection of Tamoxifen to activate Cre mediated gene expression. We expect that in the coming 10 years we will submit max. 5.1 lid1c We expect that in the coming 5 years we will submit max. 5 5.1 lid1c . **IV. Behavioral analysis upon ICV injection (5.1 lid1c ; Moderate)** To modulate the expression of target genes within our GA lines we will typical administer 5.1 lid1c We expect that in the coming 5 years we will submit max. 5.1 lid1c We expect that in the coming 5 years we will submit max. 5.1 lid1c
Gender	Most phenotypes do not differ between male and female patients. Hence we will use both sexes, but will make sure that sexes are balanced between genotypes and treatments. In the unlikely event there is a strong sex bias, we will choose the sex with the strongest phenotype.
Genetic alterations	For our research, it is vital that we use mouse models that carry mutations similar to those encountered in patients, giving the models a high construct validity which in turn translates to a high face validity.
Strain	Different standard genetic backgrounds (eg Bl6, 129) or combinations thereof (eg F1 Bl6 and 129) are used due to the sensitivity of the test paradigm to genetic background. For example the majority of our behavioural assays are carried out in a Bl6/129 background as a phenotypic difference is seen in these mice and not in pure Bl6 mice.

C. Accommodation and care
Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?
☐ Yes
☒ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices
All mice are housed and cared for in accordance with Annex III. Below are the exceptions: In a number of tests, animals will be single housed. This applies to: Nest building: These mice are single housed for a period of maximum 7 days.

5.1 lid1c

5.1 lid1c

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

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

The discomfort depends on the test and whether or not the mouse received 5.1 lid1c is used.

Surgical procedures involving 5.1 lid1c will be carried out under general anaesthesia with adequate peri and postoperative analgesia.

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

Whenever possible, mice are group-housed but some tasks, such as the nest building task, requires the mice to be single housed. The order of tasks will be planned in such a way as to minimise the length of time the mice are single housed.

For tasks that require food restriction, such as with 5.1 lid1c, mice will be maintained at 85% of their ad libitum weight.

Behavioural experiments will be carried out in the light period. This will have some effect on animal welfare, but this does not significantly add to the cumulative harm of the described tests.

With the exception of a few tasks, we do not expect the procedures to compromise the welfare of the animal beyond the distress resulting from the test. However, some of the animals are treated with a compound that could potentially have unforeseen side effects. Also, it is possible that the animals do not recover well from surgery after ICV injection.

Most GA animals will not have a harmful phenotype, but we can expect epilepsy, although this has thus far never been seen in our AS/Dup15q lines.

Explain why these effects may emerge.

This is expected to be a rare situation since the impacts of most procedures are well known, however, the therapeutics implemented are new and may elicit side effects.

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

If unexpected adverse effects are observed, immediate action will be taken. E.g., the drug dose will be reduced or treatment may be withdrawn altogether. If appropriate we will administrate pain medication or remove the animal from the experiment and euthanise it.

E. 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 F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

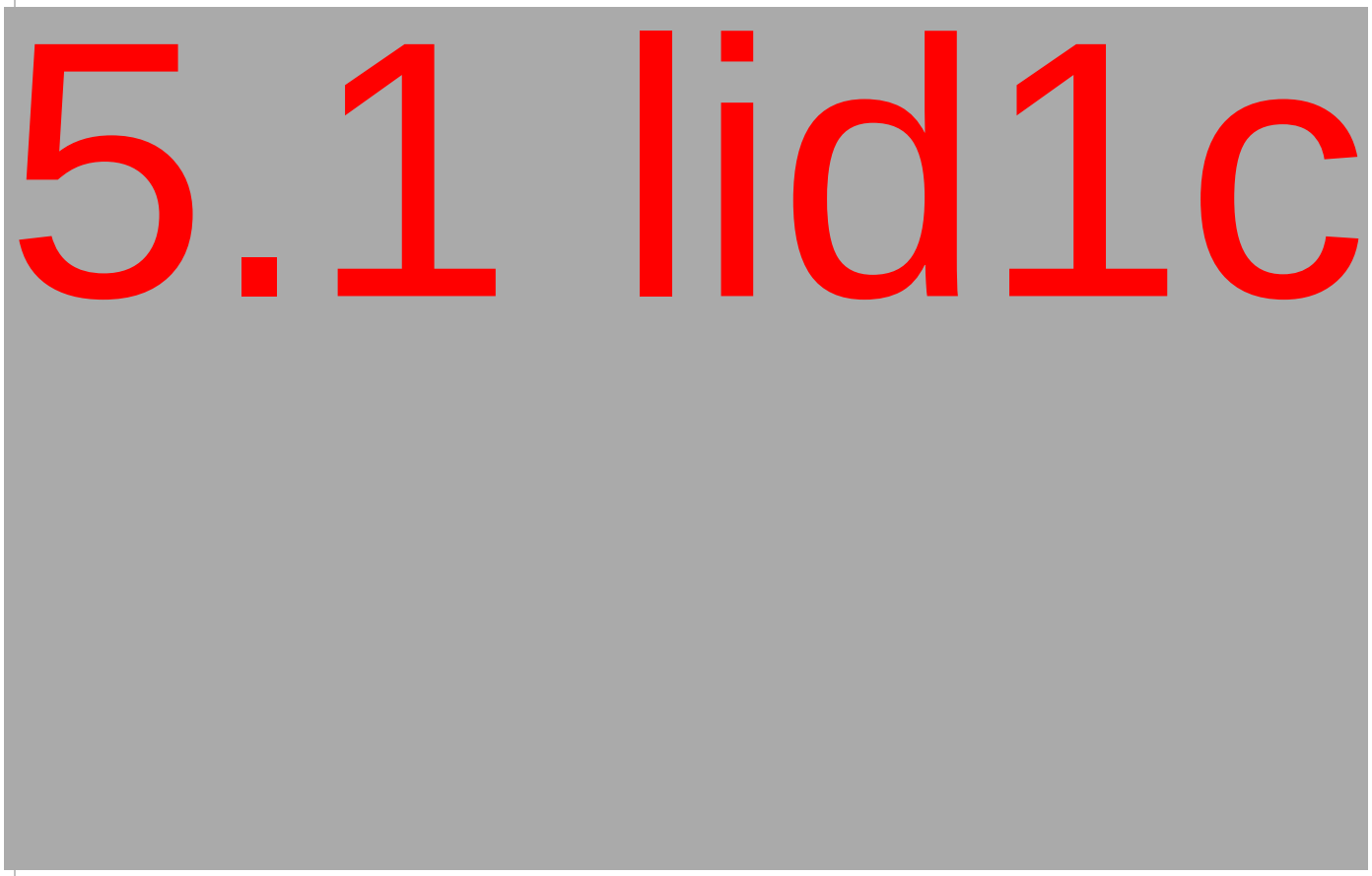
Irrespective of the cause, animals will be humanely killed in case one or more of the following criteria are met:
1. The animal stops eating or drinking.
2. Decrease of body weight of 10% relative to their weight at the start of the experiment, stunted growth before onset of the experiment (15% reduced compared to littermates).
3. For the 5.1 lid1c task, when a reduction in body weight of 20% or more occurs, the animals are removed from the experiment and fed ad libitum. If bodyweight decreases further the animals will be humanely killed.
4. Moderate circulatory or respiratory problems.
5. The development of a clinical neurological disease that results in sustained suffering beyond mild discomfort. If this involves seizures, we will terminate the animal when more than 10 seizures a day shorter than 30 seconds, or 2 seizures lasting more than 2 minutes per day, we will discontinue breeding that line. Also, we will terminate the mice if >20 seizures/day are observed in a seizure study.

Indicate the likely incidence.

According to our extensive experience with UBE3A related GA lines in the last 20 years, it is very rare to observe these events. We estimate this to be less than 1%.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in



Cumulative discomfort will never exceed moderate

Mice GA

5.1 lid1c

G. 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	In order to gain insight into the role of a gene in brain development and function, it is essential to study the effect of the genetic modification on brain development and brain function. To date, studying GA mice is the only reliable method to investigate the function of a gene in brain development and brain function *in vivo*.
Reduction	Before we embark on behavioral studies, we perform molecular and or electrophysiological studies. Only when these studies have resulted in significant interesting outcomes, we will initiate behavioral studies. This reduces the number of animals. When we use tool compounds to manipulate gene expression, they are first tested extensively in vitro before being taken along in ex-vivo experiments (neuronal cultures). Only when a tool compound is found to function well in in vitro and ex-vivo experiments, it will be tested in vivo, which reduces the number of animals we use. In addition, we perform molecular studies (which require less animals and are less invasive) before we decide to do behavioral experiments.
Refinement	The researchers keep abreast of the latest developments in this area of research through national and international collaboration with other research groups that conduct research on the genetic background of Angelman syndrome, and participate in scientific meetings. Also we check through extensive literature searches (Medline, Current Contents) that no suitable alternatives are available for a particular project. During our on-going research, intensive literature surveys will continue in search of new developments concerning alternative approaches to be implemented in this study.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☒ No > Continue with question I.

☐ 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.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

N/A

J. Location where the animals procedures are performed

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

☒ No > Continue with question K.

☐ 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.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

All mice will be killed at the end of the experiment to allow for tissue isolation and analysis.

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

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

☒ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☒ Yes > Describe the method of killing that will be used and provide justifications for this choice.

Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia. This causes least pain and stress for the animals

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Animals are only killed for scientific reasons or to resolve welfare concerns

NIET-TECHNISCHE PROJECTSAMENVATTING

Naam van het project	Mechanismen die ten grondslag liggen aan Angelman Syndroom
NTS-identificatiecode	NTS-NL-854967 v.1
Nationale identificatiecode van de NTS *Veld wordt niet gepubliceerd.*	NTS202216352
Land	Nederland
Taal	nl
Indiening bij EU *Veld wordt niet gepubliceerd.*	ja
Duur van het project, uitgedrukt in maanden.	60
Trefwoorden	Angelman syndroom Muismodellen verstandelijk handicap DUP15Q
Doel(en) van het project	Fundamenteel onderzoek: Zenuwstelsel

DOELSTELLINGEN EN VERWACHTE VOORDELEN VAN HET PROJECT

Beschrijf de doelstellingen van het project (bijvoorbeeld het aanpakken van bepaalde wetenschappelijke onduidelijkheden, of wetenschappelijke of klinische behoeften).	Angelman syndroom (AS) en Dup15q syndroom zijn ernstige neurologische aandoeningen die bij ongeveer 1: 20.000 geboorten voorkomt. Deze aandoeningen resulteren in een ernstige ontwikkelingsachterstand, verstandelijke handicap, motorische stoornissen, gedragsafwijkingen waaronder autisme, een verstoorde slaapcyclus, en de afwezigheid van spraak. Een groot aantal patiënten heeft epilepsie. Dup15q patiënten kunnen aan deze aanvallen overlijden. Angelman syndroom wordt voornamelijk veroorzaakt door verlies van het 15q11-13-gebied op chromosoom 15, terwijl Dup15q syndroom juist veroorzaakt wordt door een duplicatie van ditzelfde chromosomale gebied. Voor beide aandoeningen staat vast dat het UBE3A gen, dat in dit 15q11-13 gebied ligt, essentieel is voor het ontstaan van de symptomen. Dit voorstel richt zich daarom specifiek op het verkrijgen van inzicht in de rol van het UBE3A gen in de hersenontwikkeling. We kijken daarbij naar de functie van het UBE3A eiwit, en wat het effect is van geen of juist te veel UBE3A, op de ontwikkeling van de hersenen en het ontstaan van epilepsie. Ook willen we graag weten welke hersengebieden daarbij precies betrokken zijn. We kijken daarnaast ook naar de andere genen in het 15q11-13 gebied. We hopen dat de kennis die we vergaren uiteindelijk leidt tot een betere behandeling van de AS en DUP15q patiënten.
Welke potentiële voordelen kan dit project opleveren? Leg uit hoe de wetenschap vooruit kan worden geholpen of mensen, dieren of het milieu uiteindelijk voordeel kunnen hebben bij het project. Maak, waar van toepassing, een onderscheid tussen voordelen op korte termijn (binnen de looptijd van het project) en voordelen op lange termijn (die mogelijk pas worden bereikt nadat het project is afgerond).	We hopen dat ons onderzoek leidt tot een beter begrip van hoe het UBE3A eiwit functioneert en wat de effect is van veranderingen in de expressie van omliggende genen. Dit kan leiden tot nieuwe behandelingen voor kinderen met Angelman syndroom of Dup15q syndroom. We hebben eerder laten zien dat we bevindingen in muizen kunnen vertalen naar de kliniek, zoals een genetische therapie voor de behandeling van kinderen met het Angelman syndroom, die momenteel wordt uitgevoerd.

VOORSPELDE SCHADE

In welke procedures worden de dieren gewoonlijk gebruikt (bijvoorbeeld injecties, chirurgische procedures)? Vermeld het aantal en de duur van deze procedures.	Voor de generatie en/of import van nieuwe lijnen en voor het fokken van muizen met een licht ongerief hebben we het volgende nodig: - Moeders die fungeren als ei donoren worden geïnjecteerd met hormonen om meer eitjes te produceren (aantal 160) - Mannen worden dmv een operatie gesteriliseerd (10; deze dieren worden niet meegeteld ivm hergebruik uit een ander experiment) - Muizen die als draagmoeders fungeren worden een keer geopereerd (aantal 30) - Karakterisering van de eerste en tweede generatie muizen (aantal 200) - Fokken van muizen met een licht fenotype als gevolg van een genetische verandering (aantal 500) Verzamelen van muizenweefsel - Opzetten van neuronale culturen met hersenen uit embryo's. Moeders worden verdoofd en gedood (aantal 200), embryo's worden uit de buik gehaald en gelijk gedood zonder behandeling. Het hersenweefsel van de embryo's wordt gebruikt (aantal 1000). - Muizen van verschillende leeftijden worden onder verdoving gedood om weefsels te verzamelen voor diverse proeven (aantal 2576). Geavanceerde Karakterisering van muizen. - Muizen worden blootgesteld aan diverse gedragstaken (2260), zoals het laten lopen op een roterend loopwiel, het vermogen om een nest te maken van papier; het begraven van knikkers; het meten van exploratief gedrag in een open arena; de aanwezigheid van bepaalde reflexen en de gevoeligheid om een epileptische aanval te krijgen na blootstelling aan een hard geluid gedurende 20 seconden. - Muizen worden geopereerd waarbij elektrodes worden geplaatst waarmee epilepsie wordt gemeten ('EEG' meting) (aantal 840)
Wat zijn de verwachte gevolgen/nadelige effecten voor de dieren, bijvoorbeeld pijn, gewichtsverlies, inactiviteit/verminderde mobiliteit, stress, abnormaal gedrag, en wat is de duur van die effecten?	Generatie en import nieuwe lijnen en fokken met ongerief: Over het algemeen hebben de muizen niet al te veel last van de behandelingen. De operaties worden gedaan onder narcose en pijnstilling en de dieren zijn binnen een paar uur weer actief. Omdat we hier te maken hebben met genetisch gemodificeerde muizen is het mogelijk dat sommige nieuwe lijnen een mild fenotype vertonen. We schatten in dat maximaal 10% van de experiment muizen hier last van heeft. Dit kan variëren van een milde motorische afwijking tot epileptische aanvallen. Verzamelen van muizenweefsel: Voor een aantal dieren (moeders en embryo's neuronale culturen) worden de muizen gedood zonder voorafgaande (be)handeling. De overige dieren worden behandeld via injecties of operatief behandeld onder narcose en postoperatieve pijnstilling. Gedragsexperimenten: Muizen worden onderworpen aan een of meerdere gedragstaken. De meeste van deze taken zijn niet stressvol voor de dieren. Soms is individuele huisvesting nodig, wat stressvol is voor de muizen. De duur hiervan wordt zo kort mogelijk gehouden. Ook voor bepaalde taken is het nodig om de dieren te onderwerpen aan voedseldeprivatie, om ze te motiveren om voedsel als beloning te zien. Een ander voorbeeld van een stressvolle taak is de audiogene epilepsie test waarbij muizen gedurende maximaal 20 seconden blootgesteld worden aan een hard (±100dB) geluid. Bij sommige mutantmuizen lijnen leidt dit tot een epileptische aanval. Muizenlijnen waarvan verwacht wordt dat ze gevoelig zijn voor spontane epileptische aanvallen worden voorzien van een (draadloos) EEG meetinstrument. Hiermee wordt de hersenactiviteit gemeten dmv elektrodes die operatief geplaatst worden in aanwezigheid van postoperatieve pijnstilling. De hersenactiviteit wordt voor een periode van maximaal 2 weken gemeten.
Welke soorten en aantallen dieren zullen naar verwachting worden gebruikt? Wat zijn de verwachte ernstgraden en de aantallen dieren in elke ernstcategorie (per soort)?	

Soort:	*Totaal aantal*	*Geraamde aantallen naar ernstgraad*			
		Terminaal	*Licht*	*Matig*	*Ernstig*
Muizen (Mus musculus)	7766	0	3916	3850	0

Wat gebeurt er met de dieren die aan het einde van de procedure in leven worden gehouden?	*Soort:*	*Geraamd aantal te hergebruiken, in het habitat-/houderijsysteem terug te plaatsen of voor adoptie vrij te geven dieren*		
		Hergebruikt	*Teruggeplaatst*	*Geadopteerd*
Geef de redenen voor het geplande lot van de dieren na de procedure.	Dieren worden aan het eind van het experiment gedood en weefsels worden verzameld voor biochemische, immunocytochemische en elektrofysiologische analyses.			

TOEPASSING VAN DE DRIE V'S

1. Vervanging Beschrijf welke diervrije alternatieven op dit gebied voorhanden zijn en waarom zij niet voor het project kunnen worden gebruikt.	Een groot deel van onze onderzoeksvragen wordt beantwoord door gebruik te maken van neuronen uit stamcellen verkregen uit patiënten, daarvoor is dus geen dierproef voor nodig. Echter, om gedetailleerd inzicht te krijgen in functie van het UBE3A gen in de hersenontwikkeling, is het essentieel om een muismodel te gebruiken waarin dezelfde DNA-verandering aanwezig is als in de patiënt. Tot op heden is het bestuderen van genetisch gemodificeerde muizen de enige betrouwbare methode om de functie van een gen in hersenontwikkeling en hersenfunctie in levende dieren te onderzoeken.
2. Vermindering Leg uit hoe de aantallen dieren voor dit project zijn bepaald. Beschrijf de stappen die zijn genomen om het aantal te gebruiken dieren te verminderen en de beginselen die zijn gebruikt bij het opzetten van de studies. Beschrijf, waar van toepassing, de praktijken die gedurende het hele project zullen worden toegepast om het aantal dieren die in overeenstemming met de wetenschappelijke doelstellingen werden gebruikt, tot een minimum te beperken. Deze praktijken kunnen bijvoorbeeld bestaan uit proefprojecten, computermodellen, het delen van weefsel en hergebruik.	In het huidige PLA worden proeven gedaan op gekweekte cellen, waarmee we meerdere experimenten kunnen uitvoeren. Dit vermindert het aantal proefdieren. Het maximaal benodigde aantal dieren per experiment wordt met name bepaald op basis van eerdere experimenten. Om de aantallen laag te houden maken we daarbij zo veel mogelijk gebruik van testen die een maximaal verschil laten zien tussen mutant en controledieren. Ook realiseren we een vermindering van de benodigde aantal dieren door: - Zowel mannen als vrouwen te gebruiken wanneer de mutatie dit toelaat. - Een volgorde van verschillende testen met hetzelfde dier te kiezen, waarbij we de meeste informatie met het minste aantal dieren kunnen verkrijgen. - Geoptimaliseerde protocollen te gebruiken om de gevoeligheid van de test te maximaliseren met een minimale groepsgrootte. - Gebruik te maken van goed gedefinieerde en gestandaardiseerde genetische achtergronden.
3. Verfijning Geef voorbeelden van de specifieke maatregelen (bv. verscherpte monitoring, postoperatieve behandeling, pijnbestrijding, training van dieren) die in verband met de procedures moeten worden genomen om de welzijnskosten (schade) voor de dieren tot een minimum te beperken. Beschrijf de mechanismen om gedurende de looptijd van het project nieuwe verfijningstechnieken in gebruik te nemen.	We zullen eerst ex-vivo experimenten doen, en alleen wanneer die aanleiding geven tot vervolg onderzoek zullen we in vivo (gedrag) experimenten uitvoeren. Om stress verschijnselen tijdens de gedragsexperimenten te minimaliseren bij onze muizen worden de muizen: 1) zo veel mogelijk groep-gehuisvest 2) dagelijks 'gehandeld' om de dieren aan de onderzoekers gewend te laten raken. De onderzoekers blijven op de hoogte van de laatste ontwikkelingen op dit onderzoeksgebied door (inter)nationale samenwerking met andere onderzoeksgroepen die onderzoek doen naar de genetische achtergrond van neurologische ontwikkelingsstoornissen, en nemen deel aan wetenschappelijke bijeenkomsten. Ook controleren we door middel van uitgebreide literatuuronderzoeken (Medline, Pubmed) of er geen geschikte alternatieven beschikbaar zijn voor een bepaald project. Tijdens ons lopende onderzoek zal intensief literatuuronderzoek worden voortgezet op zoek naar nieuwe ontwikkelingen met betrekking tot alternatieve benaderingen die in deze studie moeten worden geïmplementeerd.
Licht de keuze van de soorten en de bijbehorende levensstadia toe	Muizen in alle stadia (vanaf embryo, pasgeborenen tot volwassenen (max. 8 maanden)) zullen worden onderworpen aan verschillende analyses om inzicht te krijgen in het ziekte mechanisme. Hierdoor kunnen we de verschillende ontwikkelingsstadia van de hersenen goed bestuderen. De gekozen leeftijd hangt daarnaast af van de functionele test. Voor gedragsexperimenten dienen de muizen minimaal 6 weken oud te zijn.

VOOR EEN BEOORDELING ACHTERAF GESELECTEERD PROJECT

Project geselecteerd voor BA?	nee
Termijn voor BA	

Reden voor de beoordeling achteraf

Bevat ernstige procedures	
Maakt gebruik van niet-menselijke primaten	
Andere reden	
Toelichting van de andere reden voor de beoordeling achteraf	

AANVULLENDE VELDEN

Nationaal veld 1 *Veld wordt niet gepubliceerd.*	
Nationaal veld 2 *Veld wordt niet gepubliceerd.*	
Nationaal veld 3 *Veld wordt niet gepubliceerd.*	
Nationaal veld 4 *Veld wordt niet gepubliceerd.*	
Nationaal veld 5 *Veld wordt niet gepubliceerd.*	
Startdatum project *Veld wordt niet gepubliceerd.*	
Einddatum project *Veld wordt niet gepubliceerd.*	
Goedkeuringsdatum project *Veld wordt niet gepubliceerd.*	
ICD-code 1 *Veld wordt niet gepubliceerd.*	
ICD-code 2 *Veld wordt niet gepubliceerd.*	
ICD-code 3 *Veld wordt niet gepubliceerd.*	
Link naar de eerdere versie van de NTS buiten het EC-systeem	

Centrale Commissie Dierproeven

Advies aan CCD

Datum 13 maart 2023
Betreft Advies Secretariaat over Aanvraag projectvergunning Dierproeven AVD202216352

Instelling:	5.1 lid2h
Onderzoeker:	5.1 lid2e
Project:	Mechanisms underlying the pathophysiology of Angelman Syndrome
Aanvraagnummer:	AVD202216352
Betreft:	Nieuwe aanvraag
Categorieën:	Fundamenteel onderzoek

1 Inzicht in aanvraag en de eventuele knelpunten en risico's
Deze aanvraag is minder diepgaand getoetst vanwege een kwalitatief goed DEC-advies.

Proces	De volgende vragen zijn gesteld aan de aanvrager: - Het totale aantal aangevraagde dieren in de bijlagen komt niet overeen met het aantal in de NTS. Kunt u de aantallen nalopen en de documenten met elkaar in overeenstemming brengen? - Een deel van uw aanvraag behelst het opzetten van nieuwe genetisch gewijzigde muizenlijnen. De CCD heeft afgelopen december een nieuwe handreiking op de website gepubliceerd die vanaf 1 januari van kracht is. Kunt u uw aanvraag in lijn brengen met deze handreiking? - In bijlage 2 lijkt de berekening voor het aantal dieren in experiment I niet te kloppen (tekst onder de tabel in sectie B). Kunt u nagaan of het aantal aangevraagde dieren klopt met de berekeningen? - In bijlage 2 onder K mist de onderbouwing waarom het noodzakelijk is de dieren te doden, kunt u dit toevoegen? Over de NTS: - De NTS dient anoniem te zijn. Kunt u de verwijzing naar 5.1 lid2h verwijderen? - Het algemeen publiek heeft geen inzicht in de verschillende bijlagen, kunt u de verwijzing hiernaar veranderen?

	- in de NTS komen technische termen en vakjargon voor zoals, maar niet beperkt tot, super-ovulatie, blastocysten, fenotype en founders. Kunt u de tekst nog een keer doorlopen en moeilijke termen uitleggen of vervangen door meer toegankelijke woorden? - U gebruikt de term "mild ongerief". Kunt u dit vervangen door "licht ongerief" zodat dit in lijn is met de tabel? - Kunt u iets toelichten over de aard van de gedragstaken en het ongerief dat de dieren hierdoor ondervinden?

Naam proef	**Diersoort**	**Stam**	**Aantal dieren**	**Herkomst**
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort				
	Muizen (Mus musculus)	wildtype en genetisch gemodificeerd	890	Dieren die voor onderzoek gefokt zijn
3.4.3.2. Collection of mouse tissue				
	Muizen (Mus musculus)	wildtype en genetisch gemodificeerde dieren	3.776	Dieren die voor onderzoek gefokt zijn
3.4.3.3. Behavioural analysis of mice.				
	Muizen (Mus musculus)	genetisch gemodificeerde dieren	3.100	Dieren die voor onderzoek gefokt zijn

Gebruik van mannelijke en vrouwelijke dieren

3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt.

3.4.3.2. Collection of mouse tissue

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt. citaat: Most phenotypes do not differ between male and female patients and mice, and hence we will use both sexes. In the event there is a gender bias, we will choose the gender with the strongest phenotype.

3.4.3.3. Behavioural analysis of mice.

Muizen (Mus musculus) Er worden zowel mannelijke als vrouwelijke dieren gebruikt. Zie 3.4.3.2.

Locatie uitvoering experimenten	- Alle proeven vinden plaats in een instelling van een vergunninghouder. - Er zijn geen problemen bekend met de vergunninghouder.

2 DEC advies

DEC-advies	Citaten uit het DEC advies: C10 (huisvesting): In principe zullen alle dieren conform de eisen in bijlage III van richtlijn 2010/63/EU worden gehuisvest. De studs en de gevasectomeerde mannelijke dieren worden individueel gehuisvest. In het kader van de wetenschappelijke experimenten zullen dieren alleen voorafgaand en gedurende een aantal gedragsexperimenten (de nestbouwtest, tijdens de voerbeperkingsperiode voorafgaand aan de cognitietesten) voor enige tijd individueel gehuisvest worden. Deze huisvesting is niet conform de richtlijn, maar is vanuit het belang van het experiment voldoende onderbouwd en in termen van ongerief geclassificeerd. De commissie acht het uitvoeren van de gedragstesten in de lichtperiode ook een afwijking van de standaard huisvestingomstandigheden (zie ook C11). C11 (ongerief): Bij het genereren van nieuwe lijnen is er bij de donordieren (superovulatie), de gevasectomeerde mannen en de foster dieren sprake van cumulatief matig ongerief. Gezien de aard van de betrokken genen is het niet uitgesloten dat er bij de genetisch gemodificeerde dieren sprake zou kunnen zijn van een lijn met constitutioneel ongerief. Bij de lijnen die tot nu toe gegenereerd zijn is dit nog nooit aangetroffen. Desondanks wordt de optie open gehouden dat er bij maximaal 1 lijn voor de fok en in de experimenten gebruik gemaakt zal worden van dieren met constitutioneel maximaal gering ongerief. Dieren met een fenotype resulterend in meer dan gering ongerief (de kans hierop is zeer klein) worden direct gedood. Ten gevolge van de toedieningen en mogelijke effecten van de toegediende stoffen (voor gen-inductie en behandeling) is er voorafgaand aan het doden voor het verzamelen van weefsel kans op gering (en in een zeer beperkt aantal gevallen (<1%) matig) ongerief. De uitvoering van het grootste aantal gedragstesten gaan (inclusief een eventuele voerbeperking of de toediening van tamoxifen of 'tool compounds') niet gepaard met meer dan licht ongerief. Bij een aantal testen (de '5.1 lid1c ' , de Morris watermaze, 5.1 lid1c) is sprake van maximaal matig ongerief. In een aparte groep dieren wordt door middel van audiogene prikkels de gevoeligheid voor het optreden van epileptische verschijnselen bepaald. Ook hier is sprake van matig ongerief (vooral bepaald door implantatie van de intracerebrale afleid electroden onder anesthesie).

	Het optreden van spontane insulten wordt in beperkte mate verwacht en zal nooit resulteren in meer dan licht ongerief. De commissie gaat er vanuit dat de keuze om de gedragsexperimenten uit te voeren in de licht(niet actieve) periode ook een bron van additioneel ongerief is. De commissie heeft uitgebreid met de indieners gecorrespondeerd en met de IvD vertegenwoordiger gesproken over de situatie of en wanneer er bij de voorgestelde experimenten zich situaties zouden kunnen voordoen waarbij door een stapeling van ongerief ten gevolge van verschillende interventies (elk op zich zelf resulterend in matig ongerief) uiteindelijk sprake zou kunnen zijn van cumulatief ernstig ongerief. De conclusie hieruit was dat dit in geen enkel geval te verwachten is, gezien de temporele scheiding tussen de handelingen. Op basis hiervan is de commissie tot de conclusie gekomen dat het aangegeven ongerief (3916 dieren licht en 3850 matig) realistisch, voldoende onderbouwd en herleidbaar is ingeschat en geclassificeerd. C18 (geslachten): In de wetenschappelijke geïnitieerde experimenten wordt gebruik gemaakt van zowel mannelijke als vrouwelijke dieren. In het (onwaarschijnlijke) geval dat er een duidelijk sekse gebonden verschil in fenotype wordt gevonden zullen experimenten uitgevoerd worden in het geslacht met het sterkste fenotype. Deze situatie wordt niet verwacht. C20 (doden om niet wetenschappelijke redenen): Alle dieren in de wetenschappelijk geïnitieerde experimenten worden gedood om wetenschappelijke redenen (postmortem analyse van weefsel (vooral hersenen). Voor de studs en gevasectomeerde mannelijke dieren is sprake van continued use. De draagmoeders worden gedood in het kader van het project, hoewel niet om wetenschappelijke of welzijnsredenen. Dit is moreel problematisch, maar zeer moeilijk te vermijden omdat er geen wetenschappelijke of andere bestemming is voor deze dieren. Ethische afweging van de DEC: 1) Rechtvaardigt het belang van het verkrijgen van wetenschappelijk inzicht in de rol van het UBE3A gen en het 15q11-13 gencluster bij de normale en afwijkende hersenontwikkeling waarbij ook aangrijpingspunten voor mogelijk therapeutische interventies zouden kunnen worden geïdentificeerd, het gebruik van 7766 (deels genetisch gemodificeerde) muizen en de hiermee gepaard gaande aantasting van hun integriteit en welzijn (licht ongerief (3916 muizen), matig ongerief (3850 muizen)?

2) Bij alle dieren (7766 muizen) is sprake van een aantasting van integriteit en welzijn. Bij maximaal 3850 muizen is er risico op matig ongerief en bij 3916 muizen is er sprake van licht ongerief. Het risico op het bereiken van een humaan eindpunt wordt zeer klein geschat.
Het voorgestelde onderzoek beoogt inzicht te verkrijgen in de rol van het UBE3A gen en het 15q11-13 gencluster tijdens de normale en verstoorde hersenontwikkeling en het identificeren van het neuronale substraat hiervan.
Naast dit onmiskenbaar fundamenteel wetenschappelijk belang is voor de groep patiënten met genetisch modificaties in het UBE3A gen (en hun naaste familie en de maatschappij) het belang groot.
Hoewel dat buiten de directe scope van dit project valt zou het beschikken over adequate behandelingen voor patiënten met afwijkingen in het UBE3A gen van groot persoonlijk en maatschappelijk belang zijn.
Voor de instelling met daarbinnen 5.1 lid2h is het uitvoeren van fundamenteel wetenschappelijk onderzoek in dit onderzoeksveld van groot belang. Niet in het minst voor de snelle vertaling van de wetenschappelijke resultaten naar klinische trials en uiteindelijk toepassing in de kliniek.

3) De DEC is overtuigd dat de in dit project voorgestelde experimenten een bijdrage zullen leveren aan de kennis over de rol van het UBE3A gen op de hersenontwikkeling en het neuronale substraat dat hierbij betrokken is.
Naast dit onmiskenbaar grote fundamenteel wetenschappelijke belang zijn er uiteindelijk verschillende maatschappelijke belangen gediend met de resultaten van dit onderzoek. In eerste instantie het belang van de patiënten met afwijkingen in hun UBE3A gen. Het mogelijk reduceren van hun ziektelast dient naast een groot persoonlijke belang voor de betreffende patiënten ook een aanzienlijk breder maatschappelijk belang: de verminderde ziektelast bij de familie, de zorg en de maatschappij als geheel. Omdat voor UBE3A gen gerelateerde type ziektebeelden op dit moment nog geen therapie beschikbaar is, is uiteindelijk dit onderzoek ook voor de betrokken clinici van groot belang.
De DEC is van mening dat de directe belangen voor onderzoekers en de wetenschap en de mogelijke uiteindelijke belangen voor de betreffende patiënten, hun directe omgeving, de berokken clinici en de maatschappij, voldoende zwaar wegen om het schaden van de belangen van de proefdieren om gevrijwaard te blijven van een aantasting van hun integriteit en een licht of matige aantasting van hun welzijn te rechtvaardigen.
De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, dat het project goed is opgezet, en dat de gekozen strategie

	en experimentele aanpak kunnen leiden tot het behalen van de directe doelstellingen binnen de looptijd van het project. De brede (klinische) inbedding van het onderzoek maakt de drempel naar eventueel verder klinisch onderzoek en uiteindelijk toepassing van de verkregen kennis in de kliniek zeer laag. 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 voorkomen zal worden dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven. De DEC is van oordeel dat de hier boven geschetste belangen de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van aantasting van hun integriteit en in een deel van de dieren matig ongerief rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen de aantasting van integriteit en ongerief dat de dieren wordt berokkend, is in dat opzicht voldaan. Het DEC advies is Positief Het uitgebrachte advies is niet gebaseerd op consensus. Het uitgebrachte advies is met een meerderheid van stemmen tot stand gekomen. Er is sprake van een minderheidsstandpunt wat zich primair richt op de belangen van de belanghebbende 'de wetenschap' (in termen van kwaliteit) en de daarmee verbonden belanghebbende 'de proefdieren' (in termen van aantallen en ongerief en aantasting van integriteit). Dit minderheidsstandpunt had in algemene zin niet betrekking op het belang van fundamenteel onderzoek naar het Angelman en Dup15q syndroom, maar was meer gericht op het open karakter van de aanvraag (de startmomenten van het onderzoek, de inherente onzekerheden met betrekking tot de keuzes voor bepaalde modellen en de vrijheid bij de keuzes in het kader van minimalisatie van de aantallen dieren en het ongerief bij het opzetten van de experimenten). Daarnaast was er de opvatting dat de belangen van de proefdieren geschaad worden door niet onderbouwd rekening te houden met de aangetoonde welzijnsconsequenties bij het uitvoeren van de gedragstesten tijdens de rustperiode. De meerderheid van de commissie heeft in haar afweging betrokken dat onzekerheid (zeker op een termijn van 5 jaar) inherent is aan de uitvoering van fundamenteel wetenschappelijk onderzoek en heeft bij het hierboven aangegeven 'open karakter van het project' ook zwaar de aantoonbare ervaring en kwaliteit van de wetenschappelijke

	infrastructuur binnen de onderzoeksgroep en de instelling en de kwaliteit van de in het verleden verkregen resultaten (bijvoorbeeld in de vorm van publicaties in high ranking tijdschriften en translaties naar de kliniek) meegewogen. Voor wat betreft de uitvoering van de gedragsexperimenten in de lichtperiode. Dit betreft een zeer algemene en breed toegepaste en geaccepteerde werkwijze. De commissie sluit niet uit dat deze werkwijze (dieren in een voor hen biologisch gezien 'vreemde' situatie plaatsen) onderdeel is van het model. In de correspondentie met de indieners, haar afweging en in haar advies heeft de commissie aangeven dat deze werkwijze resulteert in additioneel ongerief en (mogelijk ook) in een aantasting van de integriteit. (zie C11 en C12).

3 Kwaliteit DEC advies

Kwaliteit DEC-advies	
Het DEC advies is helder en volledig. Er is inzicht gegeven in de vragen die gesteld zijn. Bij de beantwoording van de C vragen gebruikt u een heldere onderbouwing. De ethische afweging volgt op een logische manier uit de antwoorden op de C vragen. Het minderheidsstandpunt is op een duidelijke manier weergegeven.	

4 Inhoudelijke beoordeling

3V's

Er is in voldoende mate onderbouwd dat de doelstelling niet zonder dieren behaald kan worden en het project met zo min mogelijk dieren en zo verfijnd mogelijk wordt uitgevoerd.

Hergebruik	Er is geen sprake van hergebruik van dieren.

Naam proef	**Worden de dieren gedood?**	**Doden volgens richtlijn?**
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort	Ja	volgens de richtlijn.
3.4.3.2. Collection of mouse tissue	Ja	niet volgens de richtlijn. Citaat: Mice will be sacrificed by decapitation or cervical dislocation after inhalation anaesthesia, or in the case neonatal pups, after rapid cooling in ice water. This causes least pain and stress for the animals
3.4.3.3. Behavioural analysis of mice.	Ja	volgens de richtlijn.

Naam proef		
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort		
Muizen (Mus musculus)	Ongerief: 21,3% Matig 78,7% Licht	
3.4.3.2. Collection of mouse tissue		
Muizen (Mus musculus)	Ongerief: 14,8% Matig 85,2% Licht	
3.4.3.3. Behavioural analysis of mice.		
Muizen (Mus musculus)	Ongerief: 100,0% Matig	

5 Samenvatting

5.2 lid1

Het betreft een vervolg op de eerder uitgegeven vergunning AVD5.1 lid2h Onder de voorgaande vergunning zijn functies van het UBE3A eiwit geïdentificeerd en zijn enkele muis modellen gegenereerd. Ook zijn er artikelen gepubliceerd en is er een data set openbaar gemaakt.

In principe worden de dieren gehuisvest en verzorgd volgens de richtlijn. In bijlage 3 wordt hier in bepaalde gevallen vanaf geweken. Voor de nest building test worden muizen maximaal 7 dagen individueel gehuisvest. Voor 5.1 lid1c worden dieren maximaal 8 uur gevast. Voor de 5.1 lid1c ondergaan muizen voedsel restrictie in die mate dat zij op een gewicht van 85% zullen zitten ten opzichte van het gewicht onder ad libitum voedselinname. 5.2 lid1 . Daarnaast worden gedragstesten uitgevoerd in de lichtfase. De DEC merkt dit ook aan als afwijking van standaard huisvestingsomstandigheden en als bron van ongerief.

Er zullen mannelijke en vrouwelijke muizen worden gebruikt. Indien wordt gemerkt dat het fenotype geslachtsafhankelijk is kan worden gekozen voor

gebruik van één geslacht. Deze situatie wordt echter niet verwacht.

In bijlage 3 zullen muizen gedragstesten ondergaan waarbij schokken worden gebruikt (5.1 lid1c) of waarin zij moeten zwemmen 5.1 lid1c en morris water maze). De keuze voor deze tests is voldoende onderbouwd. De DEC heeft met de aanvrager en IvD gesproken over de mogelijkheid dat door een opeenstapeling van handelingen sommige muizen ernstig ongerief zouden kunnen ervaren. De conclusie uit deze correspondentie was dat dit niet te verwachten is.

6 Voorstel besluit incl. voorstel geldigheidsduur van de vergunning

5.2 lid1

De ingangsdatum van de vergunning kan niet voor de verzenddatum van de beschikking zijn en zal indien van toepassing aangepast worden. Dit is ook het geval bij een voorgenomen besluit.

7 Concept beschikking voor akkoord CCD

Centrale Commissie Dierproeven

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5.1 lid2h
5.1 lid2e
5.1 lid2h

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Onze referentie
Aanvraagnummer
AVD5.1 lid2h202216352
Bijlagen
3

Datum 13 maart 2023
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte 5.1 lid2e ,

Op 19 augustus 2022 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Mechanisms underlying the pathophysiology of Angelman Syndrome" met aanvraagnummer AVD5.1 lid2h202216352. Wij hebben uw aanvraag beoordeeld.

Beslissing
Wij keuren uw aanvraag goed. Uit artikel 10a, eerste lid van de Wet op de dierproeven (hierna: de wet) volgt daarom dat het is toegestaan om uw project uit te voeren binnen de gestelde vergunningsperiode. Deze vergunning wordt afgegeven voor de periode van 13 maart 2023 tot en met 31 oktober 2027.

De onderbouwing van deze beslissing vindt u onder 'Overwegingen'.

Procedure

Advies dierexperimentencommissie
Wij hebben advies gevraagd bij de dierexperimentencommissie 5.1 lid2 (hierna: DEC). Dit advies is ontvangen op 13 februari 2023. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, derde lid van de wet.

Nadere vragen aanvrager
Op 1 maart 2023 hebben wij u om aanvullingen gevraagd. U heeft tijdig antwoord gegeven. Het verzoek om aanvullingen had betrekking op het aantal dieren, het volgen van de nieuwe handreiking over het creëren en fokken van genetisch gemodificeerde dieren, de noodzaak voor het doden van de dieren en enkele teksten in de niet-technische samenvatting. Uw reactie is betrokken bij de behandeling van uw aanvraag.

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

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. Nadat u een bezwaarschrift heeft ingediend kunt u een voorlopige voorziening vragen bij de voorzieningenrechter van de rechtbank in de vestigingsplaats van de vergunninghouder. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisende situatie.

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 vergunninghouder valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl, stuur een e-mail naar info@zbo-ccd.nl of neem telefonisch contact met ons op: 0800 789 0789.

Datum:
13 maart 2023
Aanvraagnummer:
AVD5.1 lid2h202216352

Centrale Commissie Dierproeven
namens deze:

5.1 lid2h
drs. F. Braunstahl
Algemeen Secretaris

Bijlagen:
- Projectvergunning
- 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: 5.1 lid2h

Adres:

Postcode en plaats:

Deelnemersnummer:

deze projectvergunning voor het tijdvak 13 maart 2023 tot en met 31 oktober 2027, voor het project "Mechanisms underlying the pathophysiology of Angelman Syndrome" met aanvraagnummer AVD5.1 lid2h202216352, na advies van 5.1 lid2h. De functie van de verantwoordelijk onderzoeker is 5.1 lid2e. Het besluit is gebaseerd op de volgende (aangepaste) stukken:

1 een aanvraagformulier projectvergunning dierproeven, zoals ontvangen op 19 augustus 2022
2 de bij het aanvraagformulier behorende bijlagen:
   a Projectvoorstel, zoals ontvangen op 8 maart 2023;
   b Bijlagen dierproeven
      - 3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort, zoals ontvangen op 8 maart 2023;
      - 3.4.3.2. Collection of mouse tissue, zoals ontvangen op 8 maart 2023;
      - 3.4.3.3. Behavioural analysis of mice., zoals ontvangen op 8 maart 2023;
   c Niet-technische Samenvatting van het project, zoals ontvangen op 8 maart 2023;
   d Advies van dierexperimentencommissie, zoals ontvangen op 13 februari 2023
   e De aanvullingen op uw aanvraag, zoals ontvangen op 8 maart 2023.

Naam proef	**Diersoort/ Stam**	**Aantal dieren**	**Ongerief**
3.4.3.1. Generation and breeding of (new) GA mouse lines with possible discomfort			
	Muizen (Mus musculus) / wildtype en genetisch gemodificeerd	890	21,3% Matig 78,7% Licht
3.4.3.2. Collection of mouse tissue			
	Muizen (Mus musculus) / wildtype en genetisch gemodificeerde dieren	3.776	14,8% Matig 85,2% Licht
3.4.3.3. Behavioural analysis of mice.			
	Muizen (Mus musculus) / genetisch gemodificeerde dieren	3.100	100,0% Matig

Aanvraagnummer: AVD5.1 lid2h 202216352

Geldende voorschriften

Wij wijzen u op onderstaande geldende voorschriften, die volgen uit artikel 1d, vierde lid, artikel 10, eerste lid 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.

Aanvraagnummer:
AVD5.1 lid2h202216352

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, derde lid van de wet. Uit artikel 10b, eerste lid van de wet 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, eerste lid van de wet de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven. Artikel 10b, tweede en derde lid van de wet schrijven 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 van de wet 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

Aanvraagnummer:
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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 van de wet moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13c van de wet volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13d van de wet 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.

Van: info@zbo-ccd.nl
Verzonden: vrijdag 17 maart 2023 11:34
Aan: 5.1 lid2h
Onderwerp: Terugkoppeling over projectvergunningsaanvraag AVD5.1 lid2h202216352

Geachte 5.1 lid2h ,

Op 19-08-2022 hebben wij een aanvraag voor een projectvergunning dierproeven ontvangen waarover uw DEC advies heeft uitgebracht. Het gaat om het project 'Mechanisms underlying the pathophysiology of Angelman Syndrome' met aanvraagnummer AVD5.1 lid2h202216352.

De CCD heeft de aanvrager aanvullende vragen gesteld. De aanvullingen hadden betrekking op het aantal dieren, het volgen van de nieuwe handreiking over het creëren en fokken van genetisch gemodificeerde dieren, de noodzaak voor het doden van de dieren en enkele teksten in de niet-technische samenvatting.

De CCD heeft besloten de vergunning toe te wijzen. De aanvrager en verantwoordelijk onderzoeker zijn hierover ingelicht. De beschikking is verstuurd op 14-3-2023.

Het DEC advies is helder en volledig. Er is inzicht gegeven in de vragen die gesteld zijn. Bij de beantwoording van de C vragen gebruikt u een heldere onderbouwing. De ethische afweging volgt op een logische manier uit de antwoorden op de C vragen. Het minderheidsstandpunt is op een duidelijke manier weergegeven.

Waar 5.1 lid2h doorgaans tijdig is met het indienden van de adviezen, valt het op dat voor deze aanvraag de DEC veel behandeltijd heeft gebruikt.

Mocht u vragen hebben over onze beslissing, dan kunt u uiteraard contact met ons opnemen.

Met vriendelijke groet,
Namens de Centrale Commissie Dierproeven

5.1 lid2e
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