101

Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-035

Ontvangen: 15-04-2011

DEC datum goedkeuring#	Type aanvraag [2]
29-04-2011	Nieuw / ~~Herz.versie / Pilot~~

VROM/GGONR[3]
DGM/SAS IG 07-103

LNV/CBDNR[4]

Hoofdproject	~~CARIM~~	~~NUTRIM~~	Hersenen en gedrag	~~GROW~~	~~biomaterialen~~	~~Ander UM~~	~~Geen UM~~
Deelproject	~~1. 2. 3.~~	~~1. 2. 3. 4.~~	~~1. 2. 3.~~	~~1. 2. 3.~~			

Financieel beheerder		Budgetnummer	30976220N

Titel van het onderzoek:

Hippocampal DNA methylation in aging and Alzheimer's disease

startdatum April 2011 einddatum [9] April 2013 *Duur van de proef [10]:* *12 weeks*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegd-heid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. Verantwoorde-lijk medewerker (VM) GGO[7]					
4. overige uitvoerenden				Art.9	
5.				Art.9 Art.12 Art.12	

Diergroep	1	2	3	4	.	.	.
ctrl/exp/sham	ctr	exp	ctr	exp			
Diersoort	01	01	01	01			
Stam	C57Bl6	C57Bl6	C57Bl6	C57Bl6			
Construct / mutatie ?	-	APPswe/PS1d E9	-	APPswe/PS1d E9			
Herkomst (leverancier) *	01	01	01	01			
Aantal	15	15	15	15			
Geslacht	m	m	m	m			
Dieren immuuncompetent ?	ja	ja	ja	ja			
Leeftijd/gewicht	6 months	6 months	15 months	15 months			
Doel van de proef *	32	32	32	32			
Belang van de proef *	01	01	01	01			
Toxicologisch onderzoek *	01	01	01	01			
Bijzondere technieken *	01	01	01	01			
Anesthesie *	04	04	04	04			
Pijnbestrijding *	01	01	01	01			
Mate ongerief *	03	03	03	03			
Toestand dier einde exp*	01	01	01	01			

* VHI-coderingen zie bijlage

1 Verantwoording

Aanvraag dierproef DEC-UM **(kaders zijn licht flexibel, maar het geheel is max. 5 pag. versie 2006)**

Titel: . Hippocampal DNA methylation in aging and Alzheimer's disease

1. Doel van de proef

The number of people suffering from Alzheimer's disease (AD) is sharply rising, while its underlying cause for most of the cases, is still unknown. Nowadays, there is growing evidence that epigenetic processes may play a crucial role in the cause and development of AD. Epigenetic processes are heritable changes in gene function that occur without actual changes in the sequence of the DNA.

The proposed research focuses on the relationship between age-related changes in one of the main epigenetic mechanisms, namely DNA methylation, and AD. This is done by studying alterations in DNA methylation with ageing in both normal mice as well as transgenic APP/PS1 mice that develop neurodegenerative and neurobehavioral symptoms related to AD.

The aim of the current study is to investigate changes in DNA methylation of genes in the brains of these mice. Our hypothesis is that aging is accompanied by major changes in DNA methylation of genes implicated in AD. Research into the role of epigenetic processes can shed more light on the pathophysiology of AD and may lay the foundation for improved treatment strategies for this devastating disorder.

This project is a new study, which is partly funded by the International Stichting Alzheimer's Onderzoek (ISAO).

2. Maatschappelijke relevantie en/of wetenschappelijk belang

Alzheimer's disease is the most common form of dementia and affects more than 6% of the population older than 65 years. To date, the pathophysiology of this disorder is not yet understood and no suitable treatment options are available. Recent evidence has suggested that epigenetic mechanisms may play a critical role in its course and development. The proposed project will significantly advance our knowledge on the exact role of epigenetic regulation in aging and AD, which is essential when it comes to developing related novel treatment strategies and prevention measures for age-related cognitive disorders like AD.

3. Alternatieven

Since we aim to investigate the pathophysiology of aging on the brain, a complex disorder like AD and correlations between the brain and periphery, cell culture techniques would not be able to suffice. Furthermore, this study relies on intricate cell-cell communication as well as the enormously complex mechanisms involved in gene-environment interactions. Therefore, only animal models can provide a complete understanding of the role of DNA methylation in aging and AD.

4. Ethische afweging

The researchers are convinced that the importance of the planned tudy outweighs the suffering of the animals involved. Since this investigation reflects a pure aging study, animals are left undisturbed until behavioral testing, i.e. no invasive manipulations are being used. Knowledge on the processes mediating (accelerated) aging, obtained in the present study, will eventually aid the development of improved treatment strategies for age-related cognitive disorders like AD. All effort is taken to minimize suffering as much as possible. Ultimately, the results of this study will minimize suffering of animals in future experiments.

5. Wetenschappelijke onderbouwing

The current proposal aims to examine the role of DNA methylation in aging and AD. Through chemical modifications of the DNA and associated histones, epigenetic mechanisms such as DNA methylation can influence gene transcription and are essential in determining a subject's phenotype. Interestingly, the dynamic and reversible nature of epigenetic modifications offers the potential of being targeted by e.g. pharmacological intervention strategies. In recent years, it has become increasingly clear that, besides stochastic changes in the epigenome that occur throughout life, environmental factors such as nutrition, diet, drugs, hormones, and infections modulate a person's phenotype via epigenetic mechanisms [1]. In fact, aging, which represents one of the major risk factors for AD, seems to be associated with remarkable epigenetic alterations [2]. More recently, several studies have examined the methylation status of promoter regions of genes implicated in the pathophysiology of AD in human postmortem cortical brain tissue. For example, a study by Siegmund and colleagues [3] showed that DNA methylation is dynamically regulated in the human cerebral cortex throughout the lifespan, involving differentiated neurons, and affecting a substantial portion of genes. Similarly, differences in DNA methylation were recently reported for the entorhinal and temporal neocortex of a monozygotic twin pair discordant for AD. Evidently, aging and AD are associated with complex patterns of aberrant methylation and its exact role in the course and development of AD is far from clear at this stage (for a complete overview of all evidence on altered DNA methylation in AD, see review by Chouliaras et al., 2010). In addition, those few studies that have been performed to date, particularly addressed cortical DNA methylation patterns. Although the hippocampus is an extremely important brain region regarding aging and age-related cognitive disorders like AD, little is known about the role of hippocampal DNA methylation in aging and AD.

More insight into the role of DNA methylation in aging and AD could add significantly to information on genetic variations and gene expression profiles for genes implicated in age-related cognitive disorders like AD. Further, it will eventually lead to a more targeted approach when it comes to developing novel treatment strategies and prevention measures concerning age-related cognitive disorders like AD.

Accordingly, the proposed study assesses differences in genome-wide gene expression profiles and DNA methylation patterns within the hippocampus of adult (6-months-old) and aged (15-months-old) wild-type (WT) and transgenic APPswe/PS1dE9 mice (from now and on referred as APP/PS1). These type of transgenic mice develop AD-like phenotype, by overexpressing mutant amyloid precursor protein (APP) and presenilin 1 (PS1), which lead to the deposition of amyloid plaques and development of cognitive deficits [4]. DNA methylation profiles will be linked to gene expression patterns and cognitive performance. Thus, the project will significantly advance our knowledge on the role of epigenetic regulation in aging and AD, which is essential when it comes to developing novel treatment strategies and prevention measures concerning age-related cognitive disorders like AD. As such, the proposed project will significantly accelerate developments in this respect.

The proposed study has the following <u>specific objectives</u>:

1. To assess differences in genome-wide gene expression profiles and DNA methylation patterns within the hippocampus of adult (6-months-old) and aged (15-months-old) wild-type (WT) mice. DNA methylation profiles will be linked to gene expression patterns and cognitive performance, allowing us to examine the functional implications of differences in epigenetic regulation.

2. To examine whether the obser ed age-effects (see objective 1) are different in transgenic APP/PS1mice.

3. To investigate to which extent central and peripheral (epi)genetic profiles are linked, by comparing hippocampal gene expression profiles and methylation patterns with those from peripheral blood mononuclear cells. More knowledge on their possible link may aid in the process of identifying clinical biomarkers relevant to the pathophysiology of AD

Of note, no experimental environmental exposure will be applied, i.e. animals are housed individually and left

undisturbed until behavioral cognitive testing as described in the SOPs (at 6 or 15 months of age) (**Appendix 1**). The only variable is aging. For more details on the actual experiments and procedures, see sections 7 and 8

6. Wetenschappelijke beoordeling

This protocol has been examined and approved by

. Further, the project is partly funded by the ISAO foundation.

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

For this study male APP/PS1 mice (C57BL/6 background) will be used as a model for Alzheimer's disease. This model is widely used in the Alzheimer field and has proven to develop learning and memory deficits at 6 months of age with concomitant neuropathological hallmarks (i.e. amyloid plaques) [4, 5]. All mice will be ordered from Jackson Laboratories.

Half (30) of the APP/PS1 and C57BL/5 (WT) offspring mice will be sacrificed at 6 months of age and the rest (30) at 15 months of age. Approximately half (32) will be killed by stretching followed by decapitation and the other half (28) of the animals via intracardial perfusion under deep pentobarbital anesthesia (100mg/kg). Stretching will be performed by a skilled person and in cooperation with the CPV.

7b. Sexe

For this investigation, only male mice will be used, which enables comparison with related studies and prevents possible confounding effects of the menstrual cycle at the epigenetic level.

7.c. Aantallen

Each group consists of 15 animals, half (8) of which will be used for (epi)genetic analyses and half (7) for immunohistochemistry (IHC; e.g. plaque load examination). Altogether, the study is based on a 2x2 design (genotype x age) and involves 4 experimental groups.

1. WT 6-month-old
2. WT 15-month-old
3. APP/PS1 6-month-old
4. APP/PS1 15-month-old

To determine the number of animals per group, a power calculation was made. Our most important outcome measure is the object location task, which is also the test with the highest degree of variation. We therefore based our power calculation on this test. We used the 'PS-Power and Sample Size Program' from VanderBilt to calculate the sample size with alpha=0.05; power=0.8; delta=0.25; sigma=0.22; m=1 (based on previous investigations). From the calculation, a sample size of 12 is derived for animals involved in behavioral testing. However, based on our experience with this test in mice we expect that about 3 animals will show insufficient exploratory behavior. ***Further, we estimate 10% drop-out in the 6-month-old APP/PS1 animals due to seizure activity. Since the animals arrive 1 month prior to behavioral examination we do not expect any further drop-outs.***

5 Dierproef

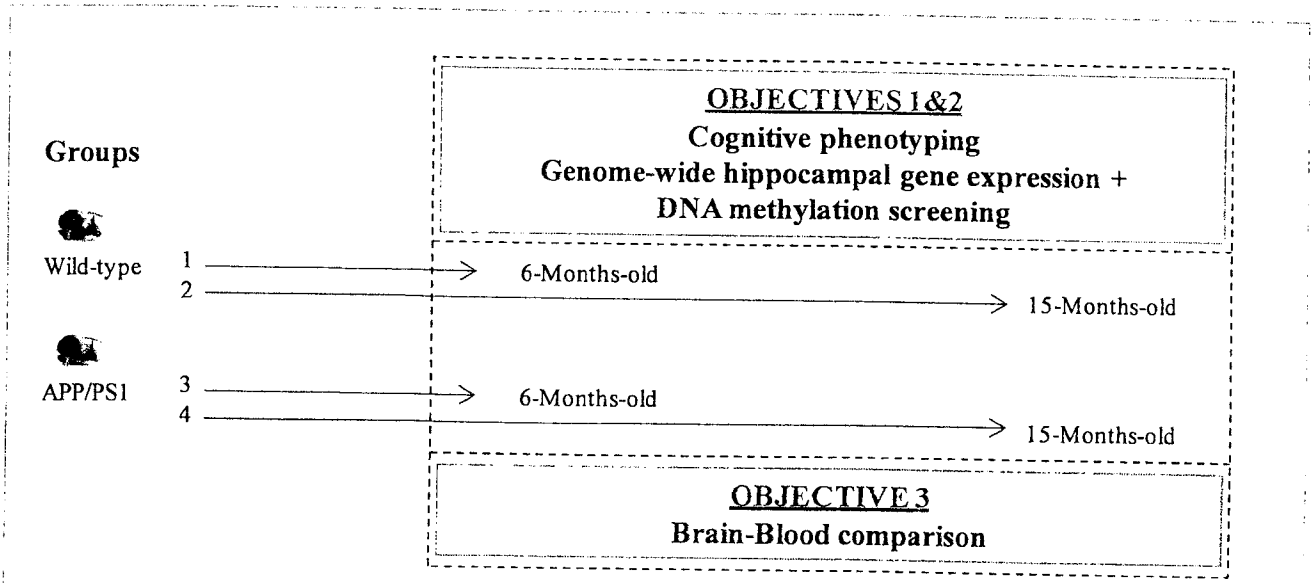
8. Experiment

Animals used for this experiment are WT and APP/PS1 mice, which have previously been shown to have elevated Aβ42 levels and amyloid lesions typical of AD by 6 months of age (Lesuisse et al., 2001, Jankowsky et al., 2004). **Animals will arrive at the CPV at an age of 5 or 14 months, respectively.** Mice will be housed **individually** within a temperature-controlled environment with a 12hr light/12hr dark cycle (lights on from 7.00 - 19.00 h) and access to standard mouse chow and water ad libitum and will be weighted weekly. Cognitive performance of the animals will be examined at 6 or 15 months of age, according to the age group. Cognitive abilities of the mice will be examined using the object recognition task, the object location task, the Y-maze and the spatial water escape task. Following behavioral testing, blood will be drawn through a vena saphenous puncture under basal conditions, for epigenetic analyses (comparison of brain and peripheral profiles).

Subsequently, half of the animals will be sacrificed by intracardial perfusion with 4% paraformaldehyde, after receiving a pentobarbital injection (100 mg/kg, i.p.). Next, the brains will be quickly removed and processed for determining amyloid plaque deposition, synaptic density and neuron numbers in the hippocampus and cortex by immunohistochemical and stereological assessment. **Altogether, the period ranging from behavioral testing to sacrifice will take 8 weeks.**

The other half of the animals will be sacrificed by stretching followed by immediate decapitation. Stretching will be performed by a skilled person and in cooperation with the CPV. Afterwards, the brain will be quickly removed from the skull. Subsequently, the brain will be microdissected to separate the hippocampus and frontal cortex. This material will be used for screening purposes on epigenetic changes using micro-array.

- ***Schematic Overview***



Group	Number	Stress Level (VHI)
WT 6-month-old	15	03
WT 15-month-old	15	03
APP/PS1 6-month-old	15	03
APP/PS1 15-month-old	15	03

All groups:

- **Mice will be housed individually upon arrival at the CPV**
- Mice will be weighted weekly
- Behavioral analysis (Y-maze , object recognition, object location test, and Morris water maze test) at an age of 6 or 15 months
- Saphenous vein puncture after the behavioral analysis , for peripheral epigenetic profiling

- Offspring will be perfused or decapitated quickly. The brains are removed and utilized for further examination.

For more details on the actual experiments and procedures see also **(Appendix 1).**

9. Experimentele condities

9a. Anesthesie

Deep pentobarbital anesthesia (100 mg/kg i.p.) will be administered after all behavioural testing on half of the mice (see section 7a). Once the animal is anesthetized, it will be sacrificed by intracardial perfusion.

9b. Pijnbestrijding

There is no reason for giving pain medication to any of the animals used.

9c. Euthanasie en Humane eindpunten

- Half of the animals are sacrificed by stretching. This will be performed by a skilled person in cooperation with the CPV. This is done without sedation, since the stress involved is known to impact on gene expression within the brain (via epigenetic mediation), which is actually one of the major outcome measures in this study. The other half will be sacrificed by intracardial perfusion under deep pentobarbital anesthesia (100mg/kg) at the 2 mentioned age points (6 and 15 months). See section 7a for more details.
- 'Humane eindpunten': During the experiment, an "ongerief dagboek' will be employed. If an animal is suffering more than expected (e.g. reduced vivacity/apathy, excessive weight loss [>15% weight loss in a week], signs of pain and/or infection, etc), a veterinarian will be contacted and, if necessary, pain medication will be applied and/or the animal will be euthanized by stretching.

Zorg

10a. Ongerief

- APPswe/PS1dE9 genotype / living with an AD-like (cognitive) dysfunction : moderate pain/stress, code 03;
- Solitary housing from 4 - or 15-months-of-age for a period of 12 weeks: moderate stress, code 03;
- Cognitive behavioral tasks: minor/moderate stress, code 02;
- Blood sampling: moderate/severe stress, code 03;
- I.P. injection before perfusion: moderate/severe stress/pain, code 03;

Exposure	*Duration*	*Frequency*	*Stress Level*
APPswe/PS1dE9 genotype	*n.a.*	*n.a.*	*03*
Solitary housing	*3 months*	*1*	*03*
Cognitive behavioral tasks	*n.a*	*1*	*02*
Blood sampling	-	*1*	*03*
I.P. injection	-	*1*	*03*

10b. Welzijnsevaluatie

Will be taken for. Data from investigations in the past: n.a.

11. Verzorging en huisvesting

The complete experiment will be performed at level (CPV). Animals will be housed individually after arrival at the CPV, to allow controlled behavioral phenotyping. The CPV will be responsible for changing cages, food and water supply. In case of problems, please contact

12. Deskundigheid

The P.I. art 9) has experience with all tests performed in mice. Where necessary he will be advised by (VVO), who is an expert in all behavioural testing paradigms, and (VM) who has experience with this mouse strain. (overige uitvoerenden) will assist in executing this project.
No non-authorized foreign employees or students are involved in this investigation.

13. Standard Operation Procedures (SOP)

See Appendix

Relevante literatuur

See Appendix

Appendix 1:
SOP's

Cognitive behavior

Novel object recognition test (ORT) and object location test (OLT)

Novel object recognition test is carried out according to previously established protocol with minor changes [6]. The apparatus consists of a circular arena, 43 cm in diameter. Half of the 40 cm high wall is made of white polyvinyl chloride, the other half of transparent polyvinyl chloride. Two objects are placed symmetrically about 5 cm away from the white wall. In the first week, the animals will be handled daily and are adapted to the procedure. In the following 2 weeks, the mice are tested until they show a good discrimination performance. A testing session is comprises of two trials (of 3 minutes each). During the first trial (T1) the apparatus contains two identical objects (samples). After the first exploration period, the mouse is put back in its home cage. Subsequently, after a predetermined delay interval (1-24h), the mouse is put back in the apparatus for the second trial (T2), but now with two dissimilar objects, a familiar one (the sample) and a new one. The times spent exploring each object during T1 and T2 is recorded manually using a personal computer. For more details, see Rutten et al. (2005). For the OLT, procedures are similar as for the ORT, with exception to the fact that no new object is introduced, but one of the (identical, familiar) samples is displayed within the arena at T2

Y-maze

The spontaneous alternation in the Y-maze was performed as previously described [7]. The symmetrical Y-maze made of acrylic consists of three arms, with all arms being apart from the others at a 120 degree angle. Each arm is 40 cm long, 17 cm high, 4 cm wide at the bottom and 13 cm wide at the top. Each mouse was placed in the centre of the Y-maze and was free to explore the arena for 6 minutes. The number of entries was counted per mouse; one entry meaning that both hind paws of the animal must be placed completely inside the arm. A mouse would be making a triad, when it visited all three arms consecutively. The measure for working memory would be the percentage of alternations that the mouse makes, being the number of triads divided by the maximum possible alternations (the total number entries minus 2) x 100.

The same maze can also be used for spatial memory. During a first trial one of three arms will be blocked and the mouse is able to explore the two open arms for 5 minutes. After a certain interval (e.g. 1 hour) the mouse will be re-introduced into the arena, but the blockade will be lifted, allowing access to the formerly closed arm. If the mouse spends more time in the novel arm compared to the old arms, it will have intact spatial memory.

Spatial Water Escape Test

The spatial water escape task represents a classic version of the Morris Water Maze task [8] and is conducted in a big pool (diameter 100 cm), filled with opaque water (21±2°C) surrounded by a set of distal and proximal spatial cues. Each daily session consists of 12 trials with a random order of start positions. A daily session includes 10 platform trials, where the platform is submerged but accessible to the mouse, as well as two probe trials in which the platform is collapsed at the bottom of the tank for variable intervals (30–40 s). At the end of the probe trial, the collapsed platform is returned to its raised position to maintain the same response-reinforcement contingency as in the platform trials. This procedure allows the use of probe trials repeatedly without the effect of extinction. The probe trials are conducted at the beginning and the end of a daily session. If the mouse fails to locate the platform in 60 s, the

experimenter will direct the mouse to the platform with his hand and the mouse will remain on the platform for 10 s. After 4 days of training, all mice are given a single probe trial after an overnight delay in order to assess the final strength of memory traces. Distance (path from the start location to the platform, cm), latency (the time to reach the platform from the start location, s), and swim speed (average speed during a trial, cm/s) are measured during the platform trials. In the probe trials, percentages of time spent in the area 40 and 20 cm in diameter around the location of platform are recorded (adapted from [9]).

Saphenous vein puncture

CPV SOP #: CPV-3-MR
Goal: Repetitive blood sampling (50µl/time) via the saphenous vein.

Materials:
- Needle (orange): 0.5mm x 16 mm, 25 G).
- Face cloth for restraining.
- Piece of gauze.
- Heparanized tubes/cups.

Preparations:
- De hind limbs are shaved one day in advance to minimize the influence of shaving-related stress on the actual experiment.
- The animal is fixed using a face cloth.
- Left or right hind limb is fixed between thumb and index finger, stretching the paw.
- If necessary, blood is driven out by forcing light pressure on the upper leg.

Blood withdrawal:
- Vein is punctured perpendicular to the surface of the skin.
- By forcing variable pressure on the upper leg the necessary amount of blood can be determined.
- Blood is collected in heparanized blood tubes.
- A new needle is used for every animal.

Aftercare:
- After the blood withdrawal bleeding is stopped by putting a piece of gauze on the wound for a few seconds.

See also
http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html

Perfusion
The animal will receive a pentobarbital injection 100mg/kg (i.p.). When the animal is under complete anesthesia (as checked for reflexes by pinching a paw), the abdominal wall and peritoneum is cut open using scissors. Secondly the diaphragm and ribs are cut open to reveal the heart and lungs. A needle connected to a pumping system is then placed in the apex of the heart and the pump is turned on to pump buffer (Tyrode) through the system. The right bosom is cut open to allow fluid to exit the vascular system.
After a 1-minute rinse with buffer, Somogyi fixation fluid (4% parafolmaldehyde, 15% picric acid, 0.05% glutaraldehyde in 0.1M phosphate buffer) is pumped through the system to ensure fixation of the tissue. After 12 minutes Somogyi fixation, the animal is decapitated and its brain is removed carefully from the skull for further processing.

Discomfort:

Forced swimming and Water maze (non-escapable water stress) are 4.
Zero maze, object recognition and location (non-escapable open field stress), marble burying and the splash test are 3.
Saphenous vein puncture: 4.
Perfusion: 4.

Reference list:

1. Chouliaras, L., et al., *Epigenetic regulation in the pathophysiology of Alzheimer's disease.* Prog Neurobiol, 2010. **90**(4): p. 498-510.
2. Fraga, M.F., R. Agrelo, and M. Esteller, *Cross-talk between aging and cancer: the epigenetic language.* Ann N Y Acad Sci, 2007. **1100**: p. 60-74.
3. Siegmund, K.D., et al., *DNA methylation in the human cerebral cortex is dynamically regulated throughout the life span and involves differentiated neurons.* PLoS One, 2007. **2**(9): p. e895.
4. Jankowsky, J.L., et al., *Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase.* Hum Mol Genet, 2004. **13**(2): p. 159-70.
5. Lesuisse, C., et al., *Hyper-expression of human apolipoprotein E4 in astroglia and neurons does not enhance amyloid deposition in transgenic mice.* Hum Mol Genet, 2001. **10**(22): p. 2525-37.
6. Rutten, K., et al., *The selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices.* Behav Brain Res, 2005. **164**(1): p. 11-6.
7. Holcomb, L., et al., *Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes.* Nat Med, 1998. **4**(1): p. 97-100.
8. Morris, R., *Developments of a water-maze procedure for studying spatial learning in the rat.* J Neurosci Methods, 1984. **11**(1): p. 47-60.
9. Savonenko, A., et al., *Episodic-like memory deficits in the APPswe/PS1dE9 mouse model of Alzheimer's disease: relationships to beta-amyloid deposition and neurotransmitter abnormalities.* Neurobiol Dis, 2005. **18**(3): p. 602-17.

University Maastricht

Faculty of Health, Medicine
and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon:

Uw referentie: Onze referentie : Maastricht, 01-03-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Hippocampal DNA methylation in aging and Alzheimer's disease*", is op de DEC vergadering van 25 februari 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De duur van de proef op het voorblad is niet juist (dit is de langste periode binnen één project dat één dier in proef is). De DEC verzoekt dit aan te passen.
- De DEC wenst een betere onderbouwing van de ethische afweging.
- Bij punt 5 verzoekt de DEC "left undisturbed" aan te passen in "individueel gehuisvest".
- Bij punt 7c verzoekt de DEC de uitval in percentages te vermelden en zonodig de berekening te herzien. De DEC vraagt zich af of de uitval voor beide groepen gelijk is.
- De DEC is van mening dat het ongerief voor solitair huisvesten code 03 is en verzoekt dit bij punt 10a aan te passen en tevens het leven met Alzheimer te vermelden.
- De duur van het solitair huisvesten dient beter en consequent aangegeven te worden, zowel bij het experiment als bij punt 10a.

Conclusie:
Het project wordt aangehouden.

Gelieve eventuele vragen te beantwoorden in **een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.**
Uw project staat bij de DEC geregistreerd onder nummer **2011-035**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Dear .

We greatly appreciate the comments made by the DEC and have addressed them in our DEC protocol. Below, you will find a point-by-point reply to the individual comments.

- De duur van de proef op het voorblad is niet juist (dit is de langste periode binnen één project dat één dier in proef is). De DEC verzoekt dit aan te passen.

The comment has been adapted.

- De DEC wenst een betere onderbouwing van de ethische afweging.

In chapter 4 the following has been added: "The researchers are convinced that the importance of the planned study outweighs the suffering of the animals involved. Since this investigation reflects a pure aging study, animals are left undisturbed until behavioral testing, i.e. no invasive manipulations are being made. Knowledge on the processes mediating (successful) aging, obtained in the present study, will eventually aid the development of improved treatment strategies for age-related cognitive disorders like AD. All effort is taken to minimize suffering as much as possible. Ultimately, the results of this study will minimize suffering of animals in future experiments."

- Bij punt 5 verzoekt de DEC "left undisturbed" aan te passen in "individueel gehuisvest".

The comment has been adapted: "Of note, no experimental environmental exposure will be applied, i.e. animals are housed individually and left undisturbed until behavioral cognitive testing as described in the SOPs (at 6 or 15 months of age)"

- Bij punt 7c verzoekt de DEC de uitval in percentages te vermelden en zonodig de berekening te herzien. De DEC vraagt zich af of de uitval voor beide groepen gelijk is.

The following has been added: "Further, we estimate 10% drop-out in the 6-month-old APPPS1 animals due to seizure activity. Since the animals arrive 1 month prior to behavioral examination we do not expect any further drop-outs".

- De DEC is van mening dat het ongerief voor solitair huisvesten code 03 is en verzoekt dit bij punt 10a aan te passen en tevens het leven met Alzheimer te vermelden.

The degrees have been adapted accordingly to code 03.

- De duur van het solitair huisvesten dient beter en consequent aangegeven te worden, zowel bij het experiment als bij punt 10a.

The comment has been adapted accordingly: "Solitary housing from 4 - or 15-months-of-age for a period of 12 weeks: moderate stress, code 03;"

Yours sincerely,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk *Doorkiesnummer* *Maastricht*
02-05-2011

Project: *Hippocampal DNA methylation in aging and Alzheimer's disease.*

Verantwoordelijk onderzoeker (VO):

Hierbij delen wij U mede dat voornoemd project aan de ethische toetsingscriteria voor proefdiergebruik voldoet.
De DEC maakt geen bezwaar tegen uitvoering van dit project zoals aangevraagd en geeft een **positief advies**.

Projectnummer:	**2011-035**
Diersoort:	**muis**
Aantal dieren:	**60**
Einddatum:	**29-04-2015**

Uw project staat bij de DEC en CPV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vice-Voorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

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