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Begeleidingsformulier aanvraag dierproef DEC- UM Herziene versie	DECNR: 2011-152 Ontvangen: 05-04-2012

DEC datum goedkeuring#	Type aanvraag [2]
16-04-2012	Nieuw / Herz.versie / Pilot

VROM/GGONR[3]
-

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	30973540E

Titel van het onderzoek:

Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)

startdatum 01-12-2011 einddatum [9] 31-12-2012 *Duur van de proef*[10]: *1 day*

	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. Verantwoordelijk medewerker (VM) GGO[7]					
4. overige uitvoerenden				Art.9	
5. **Independent scientific reviewer**				Art.9/12	

Diergroep	1	2	3.	.	.	.	.
ctrl/exp/sham	exp	exp	exp				
Diersoort	01	01	01				
Stam	WT	AD	WT				
Construct / mutatie ?	-	appsl/psl-m1461	-				
Herkomst (leverancier) *	B	A	B				
Aantal	11	17	5				
Geslacht	M	M	M				
Dieren immuuncompetent ?	ja	ja	ja	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]
Leeftijd/gewicht	20 grams	20-30 grams	20 grams				
Doel van de proef *	32	32	32				
Belang van de proef *	01	01	01				
Toxicologisch onderzoek *	01	01	01				
Bijzondere technieken *	01	17	01				
Anesthesie *	01	01	01				
Pijnbestrijding *	01	01	01				
Mate ongerief *	01	01	01				
Toestand dier einde exp*	01	01	01				

1 Verantwoording

Aanvraag dierproef DEC-UM

Titel: Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-m1461)

1. Doel van de proef.

Dementias, including Alzheimer's disease, are disabling conditions that can severely affect the quality of life for those who suffer. For many such patients incontinence and more so urgency are major additional problems which seriously affects on their care and lifestyle (Mattson, 2004; Ransmayr et al., 2008). It is generally accepted that dementia related incontinence is linked to cognitive impairment and reduced awareness. Less emphasis has been placed on possible changes in the lower urinary tract of such patients that might contribute to and exacerbate the problem. Experimental investigation of this problem in patients with dementia is difficult from moral and ethical grounds. Transgenic animals have provided an important approach for understanding the changes in dementias. Animals have been bred in which specific mutations have been engineered which mimic, in part, the changes seen in dementias including Alzheimer's disease. One such model involves a double mutation: one mutation is to the Amyloid Precursor Protein (APP) which *includes both the Swedish and London mutations (KM6706/671NL and V7171, Kohler et al., 2005) and the second to the PS1M146L mutation (PS1M146L, Duff et al., 1996). These double transgenic mice ($App_{SL}/PS1_{M146L}$) exhibit numerous changes in their central nervous system (Kohler et al., 2005; Duff et al., 1996; VanMierlo et al., 2009a, b).*
The intention of our study is to explore the idea that peripheral changes in the lower urinary tract might contribute to or exacerbate lower urinary tract dysfunction in patients with dementia. We already performed a study which focused on the distribution of nerve fibres in the bladder wall using nitric oxide synthase (nNOS) which is widely distributed in the lower urinary tract and may be important as an inhibitory neurotransmitter released by afferent nerves (Fowler et al., 2008; Hedlund, 2005).
Structural changes in bladder innervation has been shown by our group in thes alzheimer transgenic mice before (biallosterski et al 2010)
In 2010 we performed different ex vivo and in vivo experiments with bladders of guinea pig and mice(Biallosterski, van Koeveringe et al. 2011) . We have shown to be able to detect and quantify autonomous nonvoiding activity in these bladders.
We hypothesise that the nonvoiding activity in mice with an Alzheimer genotype is different from mice with a wild type genotype. We assume that the activity of the Alzheimer group will be increased Or will appear already at lower bladder volumes. In order to test this hypothesis we need to perform the ex vivo experiments in isolated bladders of both wild type mice and mice with the Alzheimer genotype.
In addition we want to make a start on investigating what differences might be expected inside the bladderwall connections, between the urothelium, submucosa and the muscle layer, and innervation using 2 foton microscopy.

2. Maatschappelijke relevantie en/of wetenschappelijk belang.

Dementias, including Alzheimer's disease, are disabeling conditions that can severely affect the quality of life for those who suffer. For many such patients incontinence and more so urgency are major additional problems which seriously affect on their care and lifestyle (Mattson, 2004;

Ransmayr et al., 2008). It is generally accepted that dementia related incontinence is linked to cognitive impairment and reduced awareness. Based on previous studies of our group,it is important to study whether there are differences in the central nervous system or also difference in the peripheral nervous system of patients (or animals) with Alzheimer's disease by means of showing differences in autonomous contractions.

3. Alternatieven

There are no alternatives to study these scientific questions besides animal experiments. The research is about the anatomy of the bladder and the behaviour of the whole organ so we can only use whole bladder models. In order to do so we need ex vivo experiments with animal bladders. We can not use strips of human material because we are at the moment interested in the movement and anatomy of the whole bladder. The experiment will be done by means of standard operating procedures which we will follow very thoroughly. We will only perform surgical procedures after euthanisation (cervical dislocatione) of the animals.
Moreover to see whether there are diffrences between the genotypes we need to do the experiments in animals with different genotypes.

4. Ethische afweging

We can not use strips of human material because we are at the moment interested in the movement and anatomy of the whole bladder.
Research using animal models is necessary for better interpretation and analysis of the neurobiological mechanisms in the bladder. We are confident that the proposed animal experiments and suffering of the animals will weight up against the new information that is going to be obtained from this study. That's why research with both animals and humans is justified in our opinion.

5. Wetenschappelijke onderbouwing

Urinary incontinence is very often a complication in dementias including Alzheimer's Disease (AD) (Mattson 2004; Ransmayr, Holliger et al. 2008). This alteration of a basic physiological process has major consequences for the quality of life of patients with AD. It is widely accepted that incontinence in dementia arises from cognitive impairment and changes in the central nervous system, which lead to a loss of conscious awareness and control of micturition. The etiology of these changes is unknown, but could be associated with the degeneration of the cholinergic innervation in the cortex and result in impaired communication with the pontine micturition centre (Yokoyama, Ootsuka et al. 2001). Furthermore the integrated physiology of the bladder itself may also contribute to this disabling condition, as structural changes are found in the bladder of a transgenic mouse model of AD (Biallosterski, de Wachter et al.). These structural changes might lead to altered peripheral sensory processing, suggesting that there could be additional mechanisms responsible for the underlying incontinence. In rodents bladder function is often studied using metabolic cages, allowing voiding frequency and volume to be measured and urine can be collected and analysed (Stechman, Ahmad et al. ; Wood, Eichel et al. 2001). However this method does not provide any information on micturition behaviour in freely moving mice. This behaviour was first studied in normal mice using a filter paper assay (Desjardins, Maruniak et al. 1973; Maruniak, Owen et al. 1974; Maruniak, Owen et al. 1975). Mice were typically placed in a cage with the bottom lined with filter paper, and afterwards urinary markings were visualised by illuminating the paper with ultraviolet light (Desjardins, Maruniak et al. 1973). Rodents, and mice in particular, use urinary marking to communicate with co specifics, about health, dominance and sexual readiness via scent (Desjardins, Maruniak et al. 1973; Maruniak, Owen et al. 1974; Maruniak, Owen et al. 1975) Frequency of urinary marking is increased when a mouse is confronted with a novel environment or other mice (Maruniak, Owen et al. 1974), whereas it is decreased upon single housing (habituation) (Arakawa, Arakawa et al. 2008). Moreover, micturition has also been related to anxiety in both animals and man (Gray 1988). In 1934, Hall first described the relationship between urine markings and 'emotionality' in rodents (Hall 1934). Further studies showed that this relationship does not appear to be influenced by loco motor activity (Milner and Crabbe 2008). Because AD is correlated to both anxiety and incontinence (Ransmayr, Holliger et al. 2008; Seignourel, Kunik et al. 2008), we conducted a preclinical study using a transgenic mouse model of AD. This study focuses on non voiding activity in mice which have a known cognitive impairment.

In the past we already studied micturition behaviour and affective an loco motor behaviour of WT and transgenic mice will be investigated using a modified filter paper assay (Desjardins, Maruniak et al. 1973; Gevaert, Vriens et al. 2007; Wood, Baez et al. 2009). Recently it has become evident that clinical problems associated with urgency, frequency and incontinence may originate from changes in both the CNS and in the periphery or end organ.

Spontaneous activity in an isolated bladder preparation of rodents has been studied by our group in the past. This activity shows close similarities with in vivo non voiding activity in the same rodents (Biallosterski, van Koeveringe et al. 2011)

Additional support for changes in innervation in the end organ, the urinary bladder, of alzheimer mice has been shown by Biallosterski et al 2010. Therefore a structured analysis of the physiological behavoir of the end organ itself wil form a logical next investigational aim.

This proposed study on AD addresses this complex issues by investigating the autonomous bladder function in an ex vivo mouse model.

We are going to investigate the AD mice via the 2-photon microscope, in order to detect the anatomy and neural innervation of the bladder and especially the lateral bladder wall. The afferent nerves are connected to the efferent nerves and we would like to show the connections. Due to Alzheimer´s disease these connections can also be changed because of different location of ganglia. That is found in mice with the Alzheimer genotype in contrast to normally aged mice (Biallosterski, 2010).

6. Wetenschappelijke beoordeling

[redacted] reviewed and approved independently by the independent

7. **Proefdier keuze**
7a. Soort, stam / herkomst / eindbestemming

Species 1: mouse
Stam: C57bl6 (wild type)
Origin: CPV/Charles River
Age: +/- 52-65 weeks
End: all animals will be sacrificed

Species 2: mouse
Stam: appsl/psl-m1461
Origin: CPV
Age: +/- 52-65 weeks
End: all animals will be sacrificed
The animals have a Alzheimer genotype so it might be possible that they suffer from having this genotype.

7b. Sexe

For the mice (both wild type and Alzheimer genotype) we need 3 couples for the breeding.
For the ex vivo experiments we only need male gender.
This is because of the longer urethra of a male mice (anatomy) so it is easier to perform the preparation and catheterize the bladder.
For immunohistochemistry experiments we are going to use both male and female.

7.c. Aantallen

Cystometry with wild type and Alzheimer mice
Based on former experiment (see DEC protocol: Structural Bladder Changes in Alzheimer's' Disease 2007 written by Bart Biallosterski) we want to include:

Wild type mice: 14
Alzheimer mice: 14
Total: 28 animals

Power calculation:
When we base the numbers of needed animals on a power calculation based on differences in amplitude and frequency between the wild type mice and the Alzheimer mice we can use the data we have from the ex vivo and in vivo experiments in the guinea pig (ref. Biallosterski 2011 and Gillespie).

The estimation on the Total number of animals is based on our previous experience on and literature review, which is expected to be on the significant effect at $\delta = 30\%$ with a standard deviation of $\sigma = 25\%$.
The significance level is $P < 0.05$ and a power of $\pi = 0.8$

Now, we will be able to determine the appropriate number of animals per group:
$N = 15.7 * (0.25/0.30)^2 = 10.9$, *e.g. 11 animals.*

Based on our previous experiences, factors such as old age of the animal and thus resulting complications like illness or death will lead to an approximately 35% loss of animal per group.
[redacted]

Two-photon microscopy with wild type mice and Alzheimer genotype mice:
Based on the fact that we will do a pilot study with two-photon microscopy we will start with 5 wild type mice to do 2 photon imaging and mapping of the bladder.

Afterwards when the results will be promising we will adjust the DEC protocol and expand the experiments with more mice and also with comparing wild type and Alzheimer genotype. Then we can make a proper power-calculation.

8. Experiment

Cystometry experiments (see also SOP 1,3)

We want to compare the autonomous bladder contractions between wild type mice and mice with an Alzheimer genotype.
Protocol:
The urinary bladder and proximal urethra of the mice will be excised microscopically (see SOP 1) immediately after cervical dislocation, and placed into Krebs' solution (mM: NaCl 121.1; KCl 1.87; $CaCl_2$ 1.2; $MgSO_4$ 1.15; $NaHCO_3$ 25; KH_2PO_4 1.17; glucose 11.0), bubbled with 5% CO_2 and 95% O_2 (pH 7.4, 30-37°C). within 15 minutes. The urethra will be cannulated with a solid plastic cannula, and secured with a fine ligature. The bladder is allowed to empty before transferring it to a 40 mL organ bath, containing 37°C constantly gassed Krebs' solution. The cannula is connected through a fluid filled tube, and a three-way connector to a pressure transducer (DTX Plus, Becton Dickinson, Franklin Lakes, NJ, USA). The transducer output will be amplified, digitized at 20 Hz and recorded using a data capture system (MP100 with AcqKnowledge 3.7.3 software, BIOPAC systems inc, California). The pressure range of this device is 0.02–180 cmH2O. The transducer will be calibrated before each experiment. Recordings start directly after applying the bladder to the organ bath.

Protocol:

A 50 minute resting period is kept before filling the bladder to 80 µl (according to experiments done in the past by Gillespie et al.). The bladder filling rate will be 80 µl/30minutes = 3µl/min. The trials start by performing a wash step directly after completion of bladder filling. This wash step is performed by emptying the organ bath, after which the organ bath is instantaneously re-filled with 100 mL of fresh Krebs' solution from below. Superfluous 60 mL Krebs' solution will be able to drain at the top of the organ bath. (see also SOP 1)

After the experiment we can use the bladders of the WT and Alzheimer mice for immunohistochemistry.

Two-photon experiment with wild type mice (afterward also with Alzheimer genotype):
The neural control of the urinary bladder within the lower urinary tract is very complex; both the parasympathetic and the sympathetic nervous system are involved. The urinary bladder itself has a triple innervation, based on the pudendus, pelvic and hypogastric nerve. Both, the pudendus and the pelvic nerve, come up at the sacral cord and the hypogastric nerve reaches the thoraco-lumbar region within the spinal cord. To control the storage and voiding of urine, several parts of the brain are important, e.g. the pontine micturition centre. But in the first instance, the innervation of the bladder and the lateral bladder wall itself, as a matter of special interest, will be examined.

Via two-photon microscopy (TPLSM) we are going to study the anatomy and neural innervation of the urinary bladder of rodents. We are going to investigate these goals with mice and guinea-pigs. I am going to start the project with tissue of wild-type mice, in an certain ex-vivo set up at the TPLSM. During these ex-vivo experiments we will use both non-stained and stained tissue, with e.g. neural and nuclei markers. Further studies could take place with an in-situ and in-vivo set up, also by means of wild-type mice. Due to the fact that our research group also works on Alzheimer

's disease it would be interesting to compare the achieved results of wild-type mice to the ones of our Alzheimer model. The mouse model we have, has the genotype APPSL/PSm146 and in former times, some studies were already done (Biallosterski et al., 2010). Immuno-histochemistry studies of Bart Biallosterski could be compared to the results of the TPLSM analysis.

All detections via TPLSM will be performed at the Department of Biomedical-Engineering (BME), under direction of . PhD. During the experiments at the BME, we are going to dissect organs and tissue of the rodents for further investigations.

The staining of the bladder tissue will be done by the following intravital markers:
Sulforhodamine B: This marker stains the elastin of the elastic fibres in red.
SytoGreen13: This marker stains the DNA/RNA in green.

After the pilot has been done we are going to use neuron markers (such as:VAChT) to map the neural innervation of the bladder.

9. Experimentele condities

9a. Anesthesie

Not applicable.

9b. Pijnbestrijding

Not applicable.

9c. Euthanasie en Humane eindpunten

All animals will be sacrificed by cervical dislocation they will be sedated with urethane on before hand.

When we see an animal is in pain or suffers during the experiment time we will euthanatize it.

If an animal will become sick we will ask the veterinary doctor of the CPV for help.

Zorg

10a. Ongerief

Due to the fact that the mice of the Alzheimer model have an Alzheimer genotype, it could be possible that they suffer from some sort of Alzheimer phenotype as well. Due to the fact that the animals will only be handled and sacrificed immediately so the degree of discomfort will be 01.

10b. Welzijnsevaluatie

In former studies there were no problems with animals as far as we know.

11. Verzorging en huisvesting

During the entire experiment, all animals will be housed in groups. The cages and water will be renewed daily by the employees of the CPV.

12. Deskundigheid

The persons involved in this experiment (see above) possess strong background of laboratory animal welfare experiences with valid licenses issued by CPV (WOD art. 9).

13. Standard Operation Procedures (SOP)

SOP1 mice (guinea pig) bladder preparation (see attachment)

Relevante literatuur

Arakawa, H., K. Arakawa, et al. (2008). "A new test paradigm for social recognition evidenced by urinary scent marking behavior in C57BL/6J mice." Behav Brain Res 190(1): 97-104.

Biallosterski, B. T., S. G. de Wachter, et al. (2010) "Changes in bladder innervation in a mouse model of Alzheimer's disease." J Chem Neuroanat 39(3): 204-210.

Biallosterski, B. T., G. A. van Koeveringe, et al. (2011). "Nonvoiding activity of the guinea pig bladder." The Journal of urology 186(2): 721-727.

Desjardins, C., J. A. Maruniak, et al. (1973). "Social rank in house mice: differentiation revealed by ultraviolet visualization of urinary marking patterns." Science 182(115): 939-941.

Gevaert, T., J. Vriens, et al. (2007). "Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding." J Clin Invest 117(11): 3453-3462.

Gray, J. A. (1988). Psychology of Fear and Stress, Cambridge University Press.

Hall, C. A. (1934). "Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality." Journal of Comparative Psychology 18(3): 385-403.

Maruniak, J. A., K. Owen, et al. (1974). "Urinary marking in male house mice: responses to novel environmental and social stimuli." Physiol Behav 12(6): 1035-1039.

Maruniak, J. A., K. Owen, et al. (1975). "Urinary marking in female house mice: effects of ovarian steroids, sex experience, and type of stimulus." Behav Biol 13(2): 211-217.

Mattson, M. P. (2004). "Pathways towards and away from Alzheimer's disease." Nature 430(7000): 631-639.

Milner, L. C. and J. C. Crabbe (2008). "Three murine anxiety models: results from multiple inbred strain comparisons." Genes Brain Behav 7(4): 496-505.

Ransmayr, G. N., S. Holliger, et al. (2008). "Lower urinary tract symptoms in dementia with Lewy bodies, Parkinson disease, and Alzheimer disease." Neurology 70(4): 299-303.

Seignourel, P. J., M. E. Kunik, et al. (2008). "Anxiety in dementia: a critical review." Clin Psychol Rev 28(7): 1071-1082.

Stechman, M. J., B. N. Ahmad, et al. "Establishing normal plasma and 24-hour urinary biochemistry ranges in C3H, BALB/c and C57BL/6J mice following acclimatization in metabolic cages." Lab Anim 44(3): 218-225.

Wood, R., L. Eichel, et al. (2001). "Automated noninvasive measurement of cyclophosphamide-induced changes in murine voiding frequency and volume." J Urol 165(2): 653-659.

Wood, S. K., M. A. Baez, et al. (2009). "Social stress-induced bladder dysfunction: potential role of corticotropin-releasing factor." Am J Physiol Regul Integr Comp Physiol 296(5): R1671-1678.

Yokoyama, O., N. Ootsuka, et al. (2001). "Forebrain muscarinic control of micturition reflex in rats." Neuropharmacology 41(5): 629-638.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon: 043

Uw referentie:

Onze referentie :

Maastricht, 24-11-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)*", is op de DEC vergadering van 18 november 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) De DEC verzoekt op het voorblad het werk- en privételefoonnummer van de vervangend verantwoordelijke onderzoeker toe te voegen.
2) Bij punt 6 verzoekt de DEC G. van Koeveringe en S. De Wachter te verwijderen. Je kunt als verantwoordelijke onderzoeker en uitvoerende van een project, niet de aanvraag zelf wetenschappelijk beoordelen. De DEC verzoekt aan te geven door wie of welke commissie dit DEC protocol wetenschappelijk is beoordeeld en goedgekeurd.
3) De vermelding van het geslacht bij punt 7b en het voorblad stemmen niet overeen. De DEC verzoekt dit aan te passen.
4) Punt 7c- De DEC verzoekt bij de berekening van de aantallen de uitleesparameter aan te geven, de uitval als percentage aan te geven en 1x te benoemen, de aantallen beter te onderbouwen en een navolgbare berekening te gebruiken en de typefout "alpha=0.005" aan te passen in "0.05".
5) Bij punt 8 in de laatste alinea, verzoekt de DEC "guinea pigs" en de zin "Further studies could, enzovoort" te verwijderen.
6) Punt 9c- De DEC verzoekt de humane eindpunten wel te vermelden, omdat er uitval wordt verwacht gezien de leeftijd van de dieren, en mogelijk vanwege deze uitval ook het ongerief aanpassen.
7) De DEC verzoekt de euthanasie bij punt 9c en de SOP in overeenstemming te brengen.
8) Bij punt 10a verzoekt de DEC het discomfort aan te passen, mogelijk door het ouder worden.

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 2011-152, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

, voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon: 043

Aan:

Uw referentie: Onze referentie : Maastricht, 19-12-2011

Geachte Onderzoeker,

De herziene versie van uw projectaanvraag: "*Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)*", is op de DEC vergadering van 16 december 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) De DEC mist de brief met de beantwoording van de vragen. Hier wordt 2x om gevraagd, (1x in de mail en 1x in de brief).
2) De DEC merkt op dat het ongerief is aangepast bij punt 10a, maar niet op het voorblad. De DEC verzoekt dit alsnog te doen.
3) Punt 7c- In de laatste zin merkt de DEC op dit "18" wildtype en "13" genetisch gemodificeerde muizen moeten zijn en verzoekt dit aan te passen.
4) De DEC wenst een motivering bij het aangepaste protocol, waarom de dropout veranderd is van 1/3 naar 1/2. Tevens wenst de DEC dat onderscheid gemaakt wordt tussen de dropout van de wildtype en de genetisch gemodificeerde muizen.
5) Punt 9a- Anesthesie wordt wel toegepast (sedatie urethaan). Codering anesthesie op het voorblad aanpassen in code 04.
6) Punt 7c: In een powerberekening wordt de delta uitgedrukt als percentage verschil tussen 2 groepen (en niet als absoluut getal), de sigma wordt ook uitgedrukt als een percentage. De DEC verzoekt de powerberekening op deze wijze aan te passen.
7) De DEC verzoekt de vragen van de DEC in een brief te beantwoorden.

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 2011-152, gelieve dit nummer in verdere correspondentie te vermelden.

De DEC-UM wenst u en uw familie fijne feestdagen en een voorspoedig en vooral gezond 2012!

Hoogachtend,

Voorzitter DEC-UM

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

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

From:

Your reference: Our reference: Maastricht, 05-01-2012

Dear

hereby the letter according to your remarks and questions of the project "*Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)*":

1) The letter according to your remarks and questions.
2) The discomfort of the mice of the Alzheimer model is both changed within the DEC protocol (chapter 10a) and the frontpage (from 01 into 02).
3) In chapter 7c the numbers of wild-type mice (18) and of genetically modified mice (13) are adjusted.
4) The drop-out of the genetically modified mice is 50%, because recent experience with the breeding colony learns that this is a safer estimate. The difference between the drop-out concerning the Alzheimer mice and the wild-type mice is due to the Alzheimer´s disease of the genetically modified mice. The dropout rate has to be differentiated between wild-type and Alzheimer mice. Wild-type is considered to be less we would take 1/3 dropout for wild-type and ½ for wild-type (so 10 wild-type mice and 13 Alzheimer mice for this experiment).
5) Referring to chapter 9a – Anesthesia: The sedation with urethane is now mentioned and the front page is adjusted, too.
6) Chapter 7c: Both delta and sigma are expressed in percentage.
7) See 1

Thank you very much for your consideration.

With best regards,

on berhalf of

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: 043

Uw referentie: Onze referentie : Maastricht, 01-02-2012

Geachte Onderzoeker,

De **herziene versie** van uw projectaanvraag: "*Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)*", is op de DEC vergadering van 27 januari 2012 besproken.

De DEC heeft één enkele vraag/opmerking:

1) Punt 7c - De DEC merkt op dat de powerberekening onnavolgbaar is. Op pagina 5, wordt aangegeven dat de sigma en delta 20% zijn. Echter deze getallen worden niet gebruikt op pagina 6. In de cursieve tekst stelt de onderzoeker vast dat met een delta van 20% de groepsgrootte n=10 dieren zou moeten zijn (echter de formule van Sachs met gebruik van sigma=20% en delta=20% leidt niet tot n=10 dieren?).
   Bovendien wordt daaronder een berekening gemaakt waarbij gebruik gemaakt wordt van andere (absolute?) getallen die leiden tot een n=6.13. De berekening van de uitval is onjuist. De DEC verzoekt een en ander te bespreken met eens statisticus en een kloppende berekening op te nemen.

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 2011-152, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon: 04

From:

Your reference: 1 1 Our reference:

Dear Mr Hoenderken,

hereby the second letter according to your remarks and questions of the project "*Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)*":

1) Point 7c: *The power calculation is adjusted. Now* The estimation on the Total number of animals is based on our previous experience on and literature review, which is expected to be on the significant effect at $\delta = 30\%$ with a standard deviation of $\sigma = 25\%$.
The significance level is $P < 0.05$ and a power of $\pi = 0.8$
Now, we will be able to determine the appropriate number of animals per group:

$$N = 15.7 * (0.25/0.30)^2 = 10.9, \text{ e.g. } 11 \text{ animals.}$$

Based on our previous experiences, factors such as old age of the animal and thus resulting complications like illness or death will lead to an approximately 35% loss of animal per group.

$$N = 11 * (100+35)/100 = 14.85, \text{ e.g. } 15 \text{ animals.}$$

Therefore, 15 animals per group will be needed in order to achieve statistical significance in our present study.

Thank you very much for your consideration.

With best regards,

on berhalf of

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

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

Uw referentie: Onze referentie : Maastricht, 03-04-2012

Geachte Onderzoeker,

De herziene versie van uw projectaanvraag: "*Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)*", is in de DEC vergadering van 30 maart 2012 besproken.

De DEC heeft één enkele vraag/opmerking:

1) De DEC constateert dat de powerberekening nog steeds niet juist is. Uitsluitend in het belang van de dieren, zal er een uitzondering worden gemaakt en is een van de DEC-leden bereid uitleg te geven over de uitvalsberekening. De DEC verzoekt de verantwoordelijke onderzoeker contact op te nemen met de secretaris van de DEC-UM (81108) om een afspraak te maken.

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 2011-152, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

From:
Sent: donderdag 5 april 2012 14:58
To:
Cc:
Subject: RE: Project 2011-152-herziene 5-4-12
Attachments: front page Mice 2011-152.doc; DEC mice 2011-152_april 2012_latest version.doc

Beste

Gisteren is overleg geweerst met over het bovengenoemde DECprotocol.
Van hem kreeg ik de toestemming om het volgens zijn aanwijzingen geadapteerde protocol samen met de frontpage tussentijds in te dienen:

hereby the second letter according to your remarks and questions of the project "*Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461)*":

1)According to your remarks and the discussion with we changed the point 7 c with the power calculation. The amount of genetic modified animals we now use for our experiments has been changed from 15 to 17. In chapter 6 we deleted the given names of the reviewer and refer to the front page.

Bij voorbaat dank voor deze mogelijkheid voor tussentijdse indiening en we hopen dat we snel de experimenten kunnen starten.

Met vriendelijke groet,

coordinator fundamental research

Maastricht University Medical Centre
P. Debeyelaan 25
P.O.Box 5800
6202 AZ Maastricht

Email:

Maastricht university

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
		16-04-2012

Project: *Ex vivo study within the isolated mice bladder concerning non-voiding activity, mapping the bladder and its neural innervation: wild-type mice vs. mice of an Alzheimer's disease model (appsl/psl-1461).*

Verantwoordelijk onderzoeker (VO):

Namens de Vergunninghouder van de DEC-UM, 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:	2011-152
Diersoort:	muis
Aantal dieren:	33
Einddatum:	16-04-2016

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

Vicevoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht