Begeleidingsformulier aanvraag dierproef DEC- UM	**DECNR: 2011-159**
Versie 2006 **Herziene versie**	**Ontvangen: 12-01-2012**

DEC datum goedkeuring#	Type aanvraag [2]
16-01-2012	Nieuw

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.	**3.**	1. 2. 3.			

Financieel beheerder		Budgetnummer	3097 3550 N

Titel van het onderzoek:

Characterization of scopolamine in an animal model of dementia

startdatum **07-02-2012** einddatum [9] **28-02-2012** *Duur van de proef [10]:* *3 wk*

	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.					

Diergroep	1	2					
ctrl/exp/sham	Exp1	Ctrl					
Diersoort	Rat	Rat					
Stam	Sprague Dawley	Sprague Dawley					
Construct / mutatie ?	-	-					
Herkomst (leverancier) *	02 (CR)	02 (CR)					
Aantal	14	7					
Geslacht	M	M					
Dieren immuuncompetent ?	ja[8]	ja[8]					
Leeftijd/gewicht	Ca 10 wk	Ca 10 wk					
Doel van de proef *	32	32					
Belang van de proef *	01	01					
Toxicologisch onderzoek *	01	01					
Bijzondere technieken *	01	01					
Anesthesie *	01	01					
Pijnbestrijding *	04	04					
Mate ongerief *	04	04					
Toestand dier einde exp*	01	01					

* VHI-coderingen zie bijlage

Verantwoording

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

Titel: Characterization of scopolamine in an animal model of dementia

1. Doel van de proef.

Scopolamine hydrobromide, a muscarinic acetylcholine receptor antagonist, is extensively used in preclinical research to induce memory dysfunction. Justification for this purpose has been provided by several studies using scopolamine as a pharmacological model for amnesia, particularly for preclinical testing of new substances designed to treat cognitive dysfunctions (Barak and Weiner 2009; Buccafusco et al., 2008; Vaisman et al., 2009; Louiseau et al., 2008). However, the underlying neuronal mechanisms are incompletely understood.
Here, we want to examine the effects of scopolamine on general neural activity, thereby gaining a better understanding of the effects of scopolamine on different brain structures.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

Memory dysfunction is a key symptom of Alzheimer's disease (AD) in patients and is part of the dementia-syndrome. AD-type dementia is often classified into two subgroups, one with early onset AD (juvenile) and one with late onset, the senile dementia. AD causes a major socio-economic burden, since it is responsible for 60% of all dementias and in the Netherlands approximately 130000 patients suffer from AD (Dutch Brain Foundation, 2010). Currently, there are no long-lasting effective therapies to stop the disease or to reduce symptoms. This project aims to evaluate a rat model of dementia in order to examine where neural inhibition takes place. Future studies will continue to identify and refine the information for further development of more effective therapeutic interventions in diseases characterized by memory impairment.

3. Alternatieven

There are no alternatives to study these scientific questions besides animal experiment. Using in-vitro conditions and computer models, it is not be possible to investigate the neurochemical changes in the brain network resulting from scopolamine application.

4. Ethische afweging

Because of the complexity of neuronal changes in the brain resulting from scopolamine injections, in-vitro experiments are not possible. Scopolamine has shown to impair memory and by finding out the exact mechanism, we can potentially identify brain regions involved in dementia-related symptoms. 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.

5. Wetenschappelijke onderbouwing

Cholinergic neurons in the CNS are believed to be involved in learning and memory (Goto et al., 1990; Bartus et al., 1982). Studies indicate that many otherwise healthy persons show a decline in cognition in later life. Animal and human studies have suggested that one major factor in age-related senile CNS dysfunction and the early states of Alzheimer's disease may be a disruption in the cholinergic neurotransmitter system (Drachman and Leavitt 1974). One model, based upon this 'cholinergic hypothesis', is provided by the use of the muscarinic receptor antagonist scopolamine (Whitehouse et al., 1982). Several studies using scopolamine as a pharmacological model for amnesia, particularly for preclinical testing of new substances designed to treat cognitive dysfunctions (Barak and Weiner 2009; Buccafusco et al., 2008; Vaisman et al., 2009; Louiseau et al., 2008).

In the present study we want to examine the effects of scopolamine on general neural activity, thereby gaining a better understanding of the effects of scopolamine on different brain structures.
If we can specify the structures which are inhibited during memory dysfunction, we could refine this information and find specific targets for our deep brain stimulation study (DEC 2011-137).

6. Wetenschappelijke beoordeling

This DEC-protocol has been scientifically reviewed and approved

4 Proefdier

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

Ten week-old Sprague Dawley male rats from Charles River (code 02) will be used. At the end of the experiments, all animals will be sacrificed (perfusion) and brains will be collected to study the mechanism of memory impairment.

7b. Sexe

Previous studies have shown that the oestrogen cycle (4-5 days) would interfere with the behaviour and biochemistry (particularly the release and uptake of dopamine and serotonin neurotransmitters) in the brain which might eventually yield some unfavorable and invalidated experimental results. Therefore, we select only the male gender as our experimental subjects in order to avoid the influences of oestrogen cycle on these neurotransmitters in the brain.

7.c. Aantallen

The estimation on the Total number of animals is based on our previous experience on immunohistochemistry and literature review, which is expected to be on the significant effect at δ = 40% with a standard deviation of σ = 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:

$N = 15.7 * (0.25/0.40)^2 = 6.13$, e.g. 7 animals per group.

As readout parameter we make use of the number c-Fos or Cytochrome C positive cells per mm^2.

Experimental groups	**Number of animals**
C-fos staining	N = 7
Cytochrome c staining	N = 7
Control	N = 7
Total	**N = 21**

5 Dierproef

8. Experiment

Procedures
Experimental groups:

Rats will be divided into 2 groups:
(1) Scopolamine
(2) Controls

Experiment
Rats will be handled for one week. Handling involves weighing and acquainting the rats to procedures of injection. Subsequently, scopolamine injections (0.1 mg/kg) will be applied once daily to the experimental group (N=14) and saline injections (0.1 mg/kg) to the control animals (N=7) for a period of 5 days. After receiving their last injection rats will be sacrificed 2h later and under deep anesthesia perfused transcardially or decapitated (1 week necessary for all rats). Brains will be removed and prepared for histologic and immunohistochemical examination. C-fos expression can be analyzed most sufficiently in perfused brains, while cytochrome c levels have to be measured in fresh brain tissue.

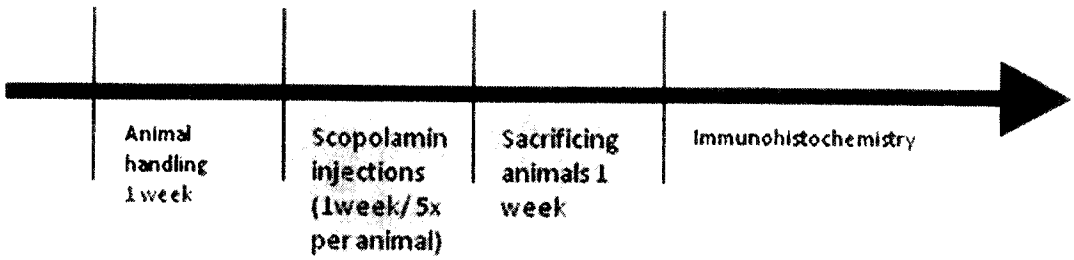

Figure 1: Chronology of the experiment.

9. Experimentele condities
9a. Anesthesie

At the end of all experiments, all animals will be sacrificed after receiving an overdose of pentobarbital.

9b. Pijnbestrijding
If animals show signs of pain or suffering the treatment with Temgesic/ Buprenorfine Hydrochloride will be provided.

9c. Euthanasie en Humane eindpunten

At the end of the experiments, all animals will be perfused or decapitated under anesthesia. If a rat becomes sick by unknown/well-known cause (for example weight loss (15%), tumor, etc.), firstly advice will be asked from the veterinary doctor of the CPV. Treatment will be conducted

according to the recommendation of a veterinary doctor. Evaluation of the health of animals will be conducted daily. If suffering of any kind appears, then euthanasia will take place.

Zorg

10a. Ongerief

In this experiment, the rats will receive repeated i.p. injections, which give the most reproducible results. In the end of experiments, animals will be sacrificed by perfusion method under deep anesthesia.

In summary, we anticipate that the total level of discomfort of the animals in this experiment is moderate.

Procedure	Week	Duration per animal	Frequency	Degree of Discomfort
Handling	Week 1	1 week	1 time / day	Code 01
i.p. injection	Week 2	1 week (5 times)	1 time / day	Code 04
Perfusion	Week 3	2 hours	1 time / day	Code 01

The experimental duration of each rat is 3 weeks.

10b. Welzijnsevaluatie

A Laboratory record logbook will be used, including the daily evaluation of each animal's welfare condition, environmental temperature and humidity.

11. Verzorging en huisvesting

During the entire experiment, all animals will be housed in pairs. The cages and water will be renewed once a week; and the weight of the animals will be measured and written down in our laboratory book.

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)

SOP perfusion

Relevante literatuur

1. Barak S, Weiner I. Towards an animal model of an antipsychotic drug-resistant cognitive impairment in schizophrenia: scopolamine induces abnormally persistent latent inhibition, which can be reversed by cognitive enhancers but not by antipsychotic drugs. The International Journal of Neuropsychopharmacology. 2009;12(02):227-41.
2. Bartus RT, Dean RLr, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science. [Review]. 1982 Jul 30;217(4558):408-14.
3. Buccafusco J, Terry A, Webster S, Martin D, Hohnadel E, Bouchard K, et al. The

scopolamine-reversal paradigm in rats and monkeys: the importance of computer-assisted operant-conditioning memory tasks for screening drug candidates. Psychopharmacology. 2008;199(3):481-94.
4. Drachman D, Leavitt J. Human memory and the cholinergic system. A relationship to aging? Arch Neurol. 1974;30(2):113-21.
5. Goto T, Kuzuya F, Endo H, Tajima T, H. I. Some effects of CNS cholinergic neurons on memory. J Neural Transm. 1990;30:1-11.
6. Loiseau F, Dekeyne A, Millan M. Pro-cognitive effects of 5-HT receptor antagonists in the social recognition procedure in rats: implication of the frontal cortex. Psychopharmacology. 2008;196(1):93-104.
7. Vaisman N, Pelled D. n- phosphatidylserine attenuated scopolamine-induced amnesia in middle-aged rats. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2009;33(6):952-9.
8. Whitehouse P, Price D, Clark A, Coyle J, DeLong M. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann Neurol. 1981;10(2):122-6.

SOP perfusie *19 november 2007*

Te gebruiken oplossingen
- Tyrode
- 4% Paraformaldehyde in fosfaatbuffer 0.1M

De rat is reeds in diepe anesthesia (urethaan)
- Geen reactie op pijnprikkel.

- Bij adequate anesthesie wordt de rat geinstalleerd op de perfusie opstelling.

- De buikwand wordt geopend.

- Het diafragma wordt geopend en het hart wordt gecanuleerd in de linkerventrikel.

Vervolgens wordt gedurende 3-4 minuten gespoeld met 150ml Tyrode om het bloed te vervangen en de kleine vaten te dilateren. Daarna wordt overgestapt naar 4% paraformaldehyde, minimaal 600ml. De hele procedure duurt 12-15 minuten.

- De canule wordt geplaatst in de linkerventrikel.
  - Start perfusie met Tyrode.
  - Rechter atrium wordt geopend.
  - Let op adequate verbleking van de lever en longen.

- Na perfusie met Tyrode, start perfusie met 4% Paraformaldehyde.
  - Let op gele verkleuring van extremiteiten en ingewanden.

- 4% Paraformaldehyde perfusie laten lopen.

- Einde Perfusie.

- Brein collectie
  - Decapitatie
  - Schedel openen voor brein collectie.
  - Brein in 4% paraformaldehyde voor post fixatie gedurende 2 uur.
  - Gevolgd door sucrose 10%, 20% en 30% over de volgende dagen voor invriezen.

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, 19-12-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Characterization of scopolamine in an animal model of dementia*", is op de DEC vergadering van 16 december 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) De DEC verzoekt het ongerief op het voorblad en bij punt 10a in overeenstemming te brengen.
2) De DEC verzoekt te bevestigen dat deze aanvraag heeft beoordeeld en goedgekeurd en tevens zijn naam en telefoonnummer naar het voorblad te verplaatsen. Het is niet de bedoeling dat verwezen wordt naar personen in de aanvraag, in verband met de Wet Openbaarheid van Bestuur.
3) De DEC is van mening dat de C-Fos bepaling en de Cytochrome, in hetzelfde dier kunnen worden uitgevoerd en dat daarmee het aantal experimentele groepen kan worden terugbracht naar 1 in plaats van 2.
4) De DEC verzoekt de tijdslijn bij punt 8 te verduidelijken, waarmee de duur van de proef duidelijk wordt. Dit moet in overeenstemming zijn met de duur van de proef op het voorblad.
5) Bij punt 10a verzoekt de DEC aan te geven dat de frequentie van de I.P. injection '5x' is.

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 **2011-159**, 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

Maastricht, 23-12-2011

Dear I

We have adopted your comments in our new DEC with the number **2011-159**.

1) *De DEC verzoekt het ongerief op het voorblad en bij punt 10a in overeenstemming te brengen.*

   We have changed the code of the severity level to 04, according to the highest degree of discomfort in this experiment.

2) *De DEC verzoekt te bevestigen dat deze aanvraag heeft beoordeeld en goedgekeurd en tevens zijn naam en telefoonnummer naar het voorblad te verplaatsen. Het is niet de bedoeling dat verwezen wordt naar personen in de aanvraag, in verband met de Wet Openbaarheid van Bestuur.*

   We have deleted name and phone number of in the DEC. He is the contact person of the Internationale Stichting Alzheimer Onderzoek, which supports this study financially. Therefore, he does not need to be mentioned on the front page and was not included in the researcher's list.

3) *De DEC is van mening dat de C-Fos bepaling en de Cytochrome, in hetzelfde dier kunnen worden uitgevoerd en dat daarmee het aantal experimentele groepen kan worden terugbracht naar 1 in plaats van 2.*

   According to our experience and the literature, c-fos expression can best be measured in perfused brain tissue, while cytochrome c is best expressed in fresh brains. Two different methods of sacrificing the animals need to be adopted and therefore we need 2 different animal groups. It was not apparent in the previous DEC version and we added this information accordingly.

4) *De DEC verzoekt de tijdslijn bij punt 8 te verduidelijken, waarmee de duur van de proef duidelijk wordt. Dit moet in overeenstemming zijn met de duur van de proef op het voorblad.*

   We gave more information about the exact timeline of this experiment. 3 weeks are needed, 1 week for handling, 1 week for scopolamine injection and 1 week for sacrificing the animals.

5) *Bij punt 10a verzoekt de DEC aan te geven dat de frequentie van de I.P. injection '5x' is.*

   We indicated at point 10a that the frequency of i.p. injection for the animals will be 5 times in total.

We hope that we have answered your questions adequately and look forward to hear from you.

Sincerely,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
[redacted]	043-	19-01-2012

Project: *Characterization of scopolamine in an animal model of dementia.*

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:	**2011-159**
Diersoort:	**rat**
Aantal dieren:	**21**
Einddatum:	**16-01-2016**

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
T (043) [redacted]

Bezoekadres

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