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Begeleidingsformulier aanvraag dierproef DEC- UM	**DECNR: 2011-137**
Versie 2006 **Herziene versie**	**Ontvangen: 16-11-2011**

DEC datum goedkeuring#	Type aanvraag [2]
16-11-2011	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:

Deep brain stimulation for memory enhancement in an experimental rat model

startdatum November 2011 einddatum [9] February 2012 *Duur van de proef[10]: 4 months*

	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	

Diergroep	**1**	**2**	.	.	.	.	.
ctrl/exp/sham	Exp	Ctrl					
Diersoort	Rat	Rat					
Stam	Sprague Dawley	Sprague Dawley					
Construct / mutatie ?	Nvt	Nvt					
Herkomst (leverancier) *	01	01					
Aantal	27	5					
Geslacht	m	M					
Dieren immuuncompetent ?	ja	Ja	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]
Leeftijd/gewicht	Ca 10 wkn	Ca 10 wkn					
Doel van de proef *	32	32					
Belang van de proef *	01	01					
Toxicologisch onderzoek *	01	01					
Bijzondere technieken *	01	01					
Anesthesie *	04	04					
Pijnbestrijding *	04	04					
Mate ongerief *	05	05					
Toestand dier einde exp*	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: Deep brain stimulation for memory enhancement in an experimental rat model

1. Doel van de proef.

The use of stimulation electrodes implanted in the brain to control severely disabling neurological and psychiatric conditions is a fast emerging area of clinical neuroscience. In line with these developments, evidence from recent clinical case studies suggests that deep brain stimulation (DBS) might enhance memory functions, when particular areas are stimulated. The primary aim of this proposal is to test the hypothesis that DBS enhances memory functions in an experimental rat model. This hypothesis is derived from key clinical case studies, which will be explained below (Hamani et al., 2008; Vignal et al., 2007). The secondary aim is to assess possible side effects of DBS on motor and non-motor behaviours. The tertiary aim is to investigate the mechanism of memory enhancement by DBS.

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 DBS as a therapy for AD or dementia-related disorders using preclinical experiments. This can be seen as a first critical step towards the application of DBS in AD/dementia patients. If DBS is effective in symptom-reduction in AD, then this could be a major breakthrough for patients, families and caregivers.

3. Alternatieven

There are no alternatives to study these scientific questions besides animal experiment. Using in-vitro conditions and computer models, it would not be possible to investigate the behavioural and neurochemical changes resulting from DBS due to the multi-synaptic connections. In addition, this research cannot be conducted in human subjects due to the following points: i. Complex behavior analysis with varying stimulation parameters in humans is not ethical, ii. With current technology it is not possible to study the changes of neurotransmitters, enzymatic activity and proteins in a non-invasive way in selected brain nuclei, iii. Electrical stimulation in healthy human subjects compare for sham-effects is not ethical.

4. Ethische afweging

Deliberation on the ethical issues of DBS in human subjects and the unavailability of human experimental brain samples restrict us to perform animal experiments. Furthermore, because of the complexity of neurobiological mechanisms of DBS, in-vitro experiments are not possible. Estimates

from 2005 suggest a global dementia prevalence of 24.3 million, with 4.6 million new cases of dementia every year. The number of people affected will double every 20 years to 81.1 million by 2040, causing an enormous economical as well as psychosocial burden. More research in the field of memory dysfunction is therefore inevitable. Deep brain stimulation within the memory circuitry has shown possible improvements and/or slowing in the rate of cognitive decline and 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

Evidence from recent clinical case studies suggests that DBS might enhance memory functions, when particular areas in the brain are stimulated. In a single-case study, DBS was performed to treat a patient with morbid obesity, but unexpectedly stimulation evoked detailed autobiographical memory events (Hamani et al., 2008). The patient reported sudden sensations that he described as "déjà vu". He reported the sudden perception of being in a park with friends, a familiar scene to him. He felt he was younger, around 20 years. He recognized his epoch-appropriate girlfriend among the people. He did not see himself in the scene, but instead was an observer. Increasing the amplitude of the stimulation resulted in side-effects such as hyperemia and sweating. Reconstruction of the electrode contact causing this phenomenon, revealed a location in the hypothalamus to the mamillary nuclei and septal areas. In the same year Vignal and coworkers showed that hallucinations of autobiographic memory could be evoked by stimulation of the amygdala, hippocampus and parahippocampal gyrus in epilepsy-patients (Vignal et al., 2007).

These clinical findings predict the existence of a memory pathway, including the hippocampus, fornix, anterior nucleus of the thalamus, hypothalamus and parahippocampal gyrus, which is also supported by animal studies (Soriano-Mas et al., 2005). Here we propose to perform DBS to modulate this memory pathway in a rat model to test the hypothesis that DBS enhances memory functions. In a previous experiment we have stimulated the hippocampal and fornix region already (see DEC 2011-040). To further evaluate the memory pathway we now want to focus on other structures of interest.

We will electrically stimulate three regions. The first region is the fimbria. The fornix/fimbria complex is very important for spatial memory as transection of the fornix/fimbria in rats abolishes both acquisition and retention of navigation to a hidden platform in the Morris water maze (Sutherland and Rodriguez, 1989). The second region will be the subiculum. The subiculum is a pivotal structure positioned between the hippocampus proper and entorhinal cortex and is thought to play a major role in working memory (Riegert et al., 2004). The final region will be the entorhinal cortex, which is part of the parahippocampal gyrus. Stone and colleagues (2011) have recenty found that acute stimulation of the entorhinal cortex (EC) transiently promoted proliferation in the dentate gyrus (DG). Cells generated as a consequence of stimulation differentiated into neurons, survived for at least several weeks, and acquired normal dentate granule cell (DGC) morphology. DBS will be performed utilizing a range of stimulation parameters (frequency, current, duration) and will be tested for the specificity of site of stimulation.

6. Wetenschappelijke beoordeling

This DEC-protocol has been scientifically reviewed and approved by the International Foundation of Alzheimer Research (ISAO). In case of concern, please contact

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 to test the hypothesis that DBS enhances memory functions and to assess its possible side effects. At the end of the experiments, all animals will be sacrificed (perfusion) for electrode localization and brains will be collected to study the mechanism of memory enhancement by DBS

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 DBS and literature review, which is expected to be on the significant effect at $\delta = 40\%$ 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:

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

The parameters of the Power Calculation are based on our previous study (DEC 2011-040). As readout parameter we make use of the exploration time of the Object Location task. Total exploration time during T1 and T2 (e1 and e2, respectively) is considered as the sum of time spent at both objects. Discrimination performance is calculated as follows (time at object at novel position – time at objects in old position)/e2, in order to correct for possible side biases. At least 7 animals are needed to reach statistical significance.

Based on our previous experiences, however, factors such as the complication of surgery and correct electrode localization will lead to an approximately 35% loss of animal per group.

$N = 6.13 * (100+35)/100 = 8.27$, e.g. 9 animals.

From our previous study (DEC 2011-040), we already collected data of sham and control animals. Half of a control group (N=5) will still be included in order to compare with previous results. No sham group will be used, since DB rats will also obtain off-stimulation conditions and therefore be equivalent to sham rats.

Experimental groups	**Number of animals**
DBS enthorhinal cortex	N = 9
DBS subiculum	N = 9
DBS fimbria	N = 9
Control	N = 5
Total	**N = 32**

8. Experiment

Procedures
Experimental groups:

Rats will be divided into 3 groups:
(1) DBS entorhinal cortex
(2) DBS fimbria
(3) DBS subiculum

From our previous study, we already collected data of sham and control animals and can spare these animals for the current experimental paradigm.

Surgery and Experiment
Animals will undergo stereotactic surgery for electrode implantation. After two weeks of recovery from surgery, rats will receive electrical stimulation in order to test for the memory functions and its side effects by DBS via a set of behavioural battery.

The implantation and electrical stimulation procedure will be conducted according to the SOP protocol (see SOP). Under general anesthesia, rats will be stereotactically implanted with a concentric bipolar electrode (tip diameter 200μm) in the brain areas of interest. The electrode will be fixed onto the skull using dental cement. After surgery, animals will receive 2 weeks of recovery. During the stimulation, electrode will be connected via a plug-in cable to the external pulse generator

The following stimulation parameters will be used
1. High frequency stimulation (100 Hz)
2. Various amplitudes (200μA, 100μA and 50μA)
3. Pulse width 100μs

Behavioural assessments
1. Memory functions (Object Location Task for spatial memory, Morris Water Maze (Ennaceur et al., 1997; Rutten et al., 2009)
2. Anxiety level (Open Field and Elevated Zero Maze)

The following behavioural tasks will be used after 2 weeks recovery from surgery:

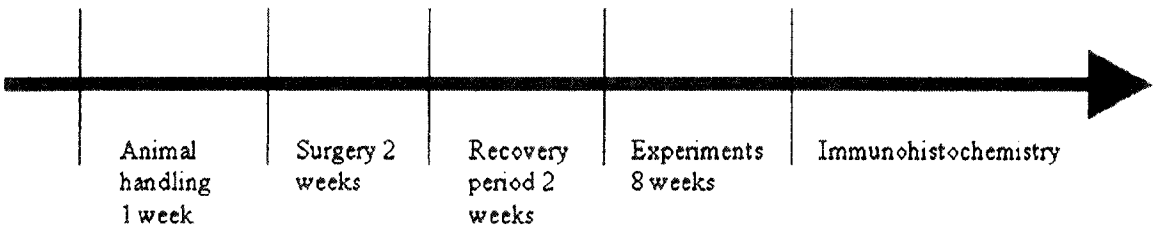

Figure 1: Chronology of the experiment.

9. Experimentele condities
9a. Anesthesie

For the surgery, inhalation anesthesia of Isoflurane will be used. Before that, the rats will receive Temgesic (0.1 mg/kg, s.c.).
At the end of all experiments, all animals will undergo perfusion. Rats will receive an overdose of pentobarbital.

9b. Pijnbestrijding
The stereotactic operation will be carried out as described in the SOP. Preoperative, lidocaine (1%) will be injected at the site of incision. In experiment, if the rat shows sign of distress after electrode implantation, animals will receive an injection with Temgesic/Buprenorfine Hydrochloride (0.01-0.05 mg/kg, s.c.) for pain reduction. The treatment with Temgesic/ Buprenorfine Hydrochloride may be repeated every 8-12 hours if animals still have signs of pain. Alternatively, Carprofen will be used for postoperative analgesia and anti-inflammatory effect.

9c. Euthanasie en Humane eindpunten

At the end of the experiments, all animals will be perfused. If a rat becomes sick by unknown/well-known cause (for example weight loss (15%) after operation, inflammation or detachment of electrodes on skull), firstly advice will be asked from the veterinary doctor of the CPV. Treatment will be conducted according to the recommendation of a veterinary doctor. If this does not result in any improvement (for responsiveness, skin infection) then the rat will be obtained from the study for further investigation. Evaluation of the health of animals will be conducted daily. If suffering is still going on, then euthanasia will take place.

Zorg

10a. Ongerief

In this experiment, the surgical procedure will be performed under inhalation anesthesia of Isoflurane with analgesic Temgesic/Buprenorfine Hydrochloride (0.1mg/kg, s.c.). In the end of experiments, animals will be sacrificed by perfusion method.

In summary, we anticipate that the total level of discomfort of the animals in this experiment is severe (code 05).

Procedure	Week	Duration per animal	Frequency	Degree of Discomfort
Handling	Week 1	1 week	1 time / day	Code 01
Surgery	Week 2-3	2-3 hours	1 time / day	Code 05
Recovery period	Week 4-5	2 weeks	1 time / day	Code 04
Object Location Task (stimulation + i.p. injection)	Week 6-8	2 weeks	1 time / day	Code 04
Morris Water Maze (stimulation + i.p. injection)	Week 9	1 week	1 time / day	Code 04
Open Field (stimulation)	Week 10	1 day	1 time / day	Code 02
Elevated Zero Maze (stimulation)	Week 10	1 day	1 time / day	Code 02
Perfusion	Week 11	1day	1 time / day	Code 01

The experimental duration of each rat is 11 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 individually in order to prevent the damage of electrode by other animals. 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 stereotactic surgery
SOP behavioural tasks

Relevante literatuur

Blokland A, Honig W, Browns F, Jolles J (1999) Cognition-enhancing properties of subchronic phosphatidylserine (PS) treatment in middle-aged rats: comparison of bovine cortex PS with egg PS and soybean PS. Nutrition 15:778-783.

Dutch Brain Foundation, 2010. Research Program Dementia. http://www.hersenstichting.nl/onderzoek/subsidies/research-programme-dementia.html.

Ennaceur A, Neave N, Aggleton JP (1997) Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Experimental Brain Research 113:509-519.

Hamani C, McAndrews MP, Cohn M, Oh M, Zumsteg D, Shapiro CM, Wennberg RA, Lozano AM (2008) Memory enhancement induced by hypothalamic/fornix deep brain stimulation. Annals of Neurology 63:119-123.

Riegert C, Galani R, Heilig S, Lazarus C, Cosquer B, Cassel JC (2004) Electrolytic lesions of the ventral subiculum weakly alter spatial memory but potentiate amphetamine-induced locomotion. Behavioural Brain Research 152:23-34.

Rutten K, Van Donkelaar EL, Ferrington L, Blokland A, Bollen E, Steinbusch HWM, Kelly PAT, Prickaerts JHHJ (2009) Phosphodiesterase Inhibitors Enhance Object Memory Independent of Cerebral Blood Flow and Glucose Utilization in Rats. Neuropsychopharmacology 34:1914-1925.

Soriano-Mas C, Redolar-Ripoll D, Aldavert-Vera L, Morgado-Bernal I, Segura-Torres P (2005) Post-training intracranial self-stimulation facilitates a hippocampus-dependent task. Behavioural Brain Research 160:141-147.

Shepherd JK GS, Fletcher A, Bill DJ, Dourish CT. (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. Psychopharmacology 116:54-64.

Stone SSD, Teixeira CM, DeVito LM, Zaslavsky K, Josselyn SA, Lozano AM, Frankland PW (2011) Stimulation of Entorhinal Cortex Promotes Adult Neurogenesis and Facilitates Spatial Memory. The Journal of Neuroscience 31:13469-13484.

Sutherland RJ RA (1989) The role of the fornix/fimbria and some related subcortical structures in place learning and memory. Behav Brain Res 32:265-277.

Vignal J-P, Maillard L, McGonigal A, Chauvel P (2007) The dreamy state: hallucinations of autobiographic memory evoked by temporal lobe stimulations and seizures. Brain 130:88-99.

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.

Standard Operating Procedure: Stereotactische operatie

Inleiding

Indien een onderzoeksvraagstelling verlangt dat een gedefinieerde kern in de rattenhersenen chemisch of farmacologisch wordt geprikkeld, of dat er een afleiding van hersenpotentialen wordt gemeten, kan dit worden gedaan met behulp van stereotaktische procedures. De rattenhersenen zijn in kaart gebracht (e.g., Paxinos & Watson, The rat brain in stereotaxic coordinates, Academic Press: Sydney, 1986). Nadat de kop van de rat in de gewenste positie in de stereotakter is gebracht, biedt het coördinatenstelsel van de hersenatlas de mogelijkheid de exacte positie van de verschillende gebieden te bepalen. De positiebepaling van de hersengebieden kan gedaan worden vanuit drie referentiepunten, i.e., het bregma, het lambda, of de interauraal lijn. Deze referentiepunten kunnen alleen gebruikt worden indien de rat tijdens de operatie gefixeerd is, en de positie van de manipulatorarm (met daaraan vastgemonteerd een, ijknaald, spuit, electrode, of canule) en het referentiepunt ten opzichte van elkaar hetzelfde blijft. De manipulatorarm is aan het fixatie gedeelte verbonden, en het totale apparaat wordt een stereotakter ('stereotactic frame') genoemd.

NB. Alle genoemde procedures zijn beschreven voor de rat, maar gelden uiteraard ook voor de muis.

Anaesthesie en analgesie bij operatie

De stereotactische operatie wordt onder algehele narcose uitgevoerd. Om de rat onder narcose te krijgen wordt gebruik gemaakt van het inhalatie anestheticum isofluraan. Dertig minuten voor de inductie met isofluraan wordt een gift buprenorphine *(0.01-0.05 mg/kg s.c. LG elke 8-12h)*gegeven voor adequate pijnstilling. Om eventuele postoperatieve pijn te bestrijden zal zo nodig buprenorfine toegediend worden. Wanneer post operatief het vermoeden bestaat dat een rat nog steeds pijn heeft, zal een extra gift buprenorfine toegediend worden.

Voordat de rat in de stereotacter gefixeerd wordt, dient de reflex die optreedt na het knijpen in de tenen verdwenen te zijn. De ogen zullen met oogzalf worden behandeld om zodanig uitdroging te voorkomen. Ook zal gekeken worden of de ademhaling diep en regelmatig is (ook na toediening van matige pijnprikkel. Aangezien het periost wordt verwijderd op de schedel, zal tevens locaal lidocaine op de wond worden toegediend.

Apparaat en procedures

De haren op de hoofdhuid worden afgeschoren en verwijderd. Daarna wordt de rat in de stereotakter gezet. Met behulp van twee stompe oorpennen, die in de gehoorgang van de rat gebracht worden, en een bekklem voor de snijtanden, wordt de kop van de rat gefixeerd.

Na desinfectie van de hoofdhuid met betadine wordt de schedel vrijgemaakt door een incisie in de hoofdhuid. Alle operatie materialen (scalpels, wondklemmen, naalden etc.) worden voor gebruik gedesinfecteerd in alcohol. De huid wordt zodanig gefixeerd dat de schedel goed bereikbaar is. Daarna wordt de schedel schoon en droog gemaakt met steriel watjes. De schedel wordt in een horizontale positie gebracht door de referentiepunten bregma en lambda op dezelfde hoogte in te stellen. Vervolgens wordt de middellijn (i.e., lijn door lambda en bregma) bepaald.

Nadat de rat in het stereotaktische frame is gefixeerd ligt het referentiepunt voor de interauraal lijn vast. Om het referentiepunt bregma/lambda te bepalen moet de ijknaald naar het bregma/lambda gebracht worden en deze coördinaten opgeschreven worden. Deze coördinaten moeten aangepast worden aan de gewenste atlascoördinaten. Dan wordt op de laterale en de anterior/posterior coördinaten de schedel doorboord tot aan de dura (diameter van boorgat 0.8 mm). Nadat het gat in de schedel is geboord wordt de dura mater met een injectienaald doorgeprikt. Vervolgens kan men de electrode naar de van te voren vastgestelde dorsoventrale coördinaat. Waarna deze middels schroeven en cement vast gezet wordt op de schedel van de rat.

SOP Gedragstaken

Open Field Test (OF): OF test is performed to measure spontaneous locomotor and exploratory activity in rats (Blokland et al 2002). The OF test is conducted in a square, clear Plexiglas box (100 cm × 100 cm × 30 cm), with an open top and a dark floor. The arena of the OF is subdivided in 'corner' (four squares each 16 cm × 16 cm), 'wall' (four rectangles each 16 cm × 64 cm) and 'center' (one square 64 cm × 64 cm) zones. The OF is placed on the floor of an experimental room. A camera is installed 2.5 m above the center of the field. Immediately after a rat is placed in the center of the OF, the movements and position of the animals are recorded and registered automatically by a computerized system (EthoVision, Noldus Equipment, The Netherlands). Only the total duration of rearing and leaning (i.e. rearing but making contact with the forepaws against the wall) are manually recorded. The illumination of the room is reduced to 60 lx on the floor of the apparatus. Testing will be carried out on 4 consecutive days. The floor of the OF is cleaned with a damp sponge after each session, which lasts 5 min, to prevent transmission of olfactory cues. OF behavior is tested between 13:30 and 17:00 h.

Zero Maze: The zero-maze, as originally described in Shepherd et al. (1994), is made of black plastic (transparent for infrared light). It consists of a circular runway (98 cm in diameter, 10 cm path width, 70 cm above floor level) which divided equally into two opposite open and two opposite parts enclosed with 50 cm high side walls. A 7 mm high edge surrounds the open parts to prevent falls. A rat is placed into one of the open parts facing a closed part and allowed to explore the maze over a period of 5 min. Total and relative duration and distance travelled in open and enclosed parts are measured under low loght conditions (1-2 lux) via an infrared video camera connected to a video tracking system (Ethovision Pro, Noldus Information Technology, Wageningen, The Netherlands). The maze is thoroughly cleaned with water after each animal. All testing is carried out between 07:30 and 12:30 h.

Object Location Task: Testing is performed in a circular arena, to which the animals are first habituated. In order to observe improvements in memory performances, rats will be injected intraperitoneally with scopolamine 30 min before the trial. Scopolamine is a muscarinic acetylcholine receptor antagonist and therefore induces memory impairment. Rats will then be stimulated shortly before and throughout the first trial. In the first trial (T1) two identical objects are placed in a symmetrical position about 10 cm away from the walls. A rat is always placed in the apparatus facing the wall at the middle of the transparent segment. After T1 the rat is put back in its home cage and the second trial (T2) follows after 90 min. In T2 one of the objects presented before is moved 10 cm to the front or back of the arena. The trials are recorded using a camera mounted above the arena and scored for the amount of time spent sniffing the objects; the object-location discrimination index is calculated (Ennaceur et al., 1997; Rutten et al., 2009).

Morris Water Maze: Performance in the Morris water maze is assessed in a water tank that consisted of a circular black tub, with a slightly sloping wall (polyethylene, inner dimensions: diameter at top 153 cm, diameter at bottom 143 cm, depth 63cm), filled with 43.5L of clear tap water at a temperature of approximately 22°C. The escape platform consists of a black polyethylene cylinder (diameter 10.8 cm) submerged 1.5ccm below the surface of the water. In this version, the water is not made opaque because a black escape platform is not visible in

a black tank. Again, in order to observe improvements in memory performances, rats will be injected intraperitoneally with scopolamine 30 min before the trial. A video camera, mounted in the center above the circular pool, provided a picture of the pool on a television monitor. The movements of a rat will be automatically registered with a computer program (EthoVision, Noldus Equipment, Wageningen, The Netherlands) (Blokland et al., 1999).

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, 26-10-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Deep brain stimulation for memory enhancement in an experimental rat model*", is op de DEC vergadering van 21 oktober 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt de ethische afweging (punt 4), toe te spitsen op het voorgestelde experiment.
- De DEC verzoekt te bevestigen dat "**dit**" DEC protocol wetenschappelijk beoordeeld en goedgekeurd is door de ISAO en een contactpersoon te vermelden.
- Punt 7c- De DEC verzoekt in de toekomst niet tussentijds af te ronden. De DEC mist de uitleesparameter, deze gaarne nog vermelden. De DEC stelt voor een halve controlegroep te includeren ter vergelijking met eerdere experimenten.

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 2011-137, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Maastricht, 07-11-2011

Dear

In the following we are going to respond to the questions of your letter from 26-10-2011 for our project with the DEC number **2011-137**.

- De DEC verzoekt de ethische afweging (punt 4), toe te spitsen op het voorgestelde experiment.

➔ We have revised punt 4 accordingly. We are certain that animal experimentation is essential in the development of important medical advances with deep brain stimulation (DBS). Deliberation on the ethical issues of DBS in human subjects and the unavailability of human experimental brain samples restrict us to perform animal experiments. If the application of deep brain stimulation in structures of the memory circuitry proves to be beneficial, this tool can be used to delay or slow the progression of memory loss in people suffering from dementia. Currently over 25 million people are diagnosed with dementia, causing an enormous economical as well as psychosocial burden. Therefore, there is an urgent need for developing and evaluating effective therapeutic interventions.

- De DEC verzoekt te bevestigen dat "dit" DEC protocol wetenschappelijk beoordeeld en goedgekeurd is door de ISAO en een contactpersoon te vermelden.

➔ We have included a contact person from the ISAO, who reviewed and approved "this" DEC protocol. We have also provided a telephone number for any further queries.

- Punt 7c- De DEC verzoekt in de toekomst niet tussentijds af te ronden. De DEC mist de uitleesparameter, deze gaarne nog vermelden. De DEC stelt voor een halve controlegroep te includeren ter vergelijking met eerdere experimenten.

➔ The calculation has been corrected.

$N = 15.7 * (0.25/0.40)^2 = 6.13$
$N = 6.13 * (100+35)/100 = 8.27$, *e.g. 9 animals per group*

We have also included half a control group (N=5) in order to compare with previous results, leading to a total number of 32 animals for this experiment.

Experimental groups	**Number of animals**
DBS enthorhinal cortex	N = 9
DBS subiculum	N = 9
DBS fimbria	N = 9
Control	N = 5
Total	N = 32

Regarding the readout parameter in the different tests we are using, we are especially interested in the exploration time the rat spends at the objects in the first and second trial of the object location task [in s]. In the open field and the elevated zero maze we are interested in the time the rat spends in different areas of the arena [in s] as well as distance moved [in cm]. In the Morris water maze we investigate the time it takes for the rat to reach the hidden platform [in s] as well as the distance moved [in cm].

We hope we have answered all your questions to your satisfaction. For any further queries, please do not hesitate to contact us.

Sincerely,

From: Dec Secretariaat
Sent: maandag 14 november 2011 8:38
To:
Subject: FW: Dec 2011-137

Attachments: DEC brief.doc; DEC aanvraag formulier (1) (1).doc; Voorblad aanvraag dierproef DEC (1).doc


DEC brief.doc (37 KB)


DEC aanvraag ormulier


Voorblad (raag dierproef I

Geachte onderzoeker, beste ,

De DEC heeft de herziene bsproken en vindt de vragen nog niet voldoende/cq. goed beantwoord.

Punt 4:
De eerste zin kan verwijderd worden, dit is niet relevant voor de DEC.
De DEC wil hier graag zien staan bijvoorbeeld: Het gebruik van dieren is noodzakelijk aangezien de complexiteit van neurobiologie-welke niet mogelijk te onderzoeken is in vitro (iets in deze trand).

Punt 7c:
Afronden is nu correct gebeurd. Echter, de uitleesparameter bij de groepen is nog steeds niet genoemd. "Regarding the readout parameter in the different tests we are using, we are especially interested in the exploration time the rats spend at the objects in the first and second trial of the object location task [in s]. In the open field and the elevated zero maze we are interested in the time the rats spend in different areas as described below [in s] as well as distance moved [in cm]. In the Morris water maze we investigate the time it takes for the rat to reach the hidden platform [in s] as well as the distance moved [in cm]." -->Er staan hier dus een aantal uitleesparameters waar de onderzoekers in geinteresseerd zijn - het moet er echter EEN zijn waar de statistiek op berust (minst gevoelige parameter die het grootste aantal dieren vereist)!!!

Graag je reactie.

Met vriendelijke groet namens DEC-UM:

Ambtelijk Secretaris Dierexperimentencommissie
MD Maastricht T
E-mail:
Werktijden: Ma-Di-Wo-Don van 08.00 uur tot 16.00 uur

Postbus 616-UNS 50-Box 48, 6200

-----Original Message-----
From:
Sent: maandag 7 november 2011 10:55
To: Dec Secretariaat
Subject: AW: Dec 2011-137

Dear DEC-UM group,
I have included the letter, in which I answered all questions from the Wijzigingsbrief. Please find all revised documents attached to this email.

Kind regards,

Maastricht, 14-11-2011

Dear

We apologize for any misunderstanding regarding your questions from your letter of 26-10-2011 for our project with the DEC number 2011-137.

We have revised point 4 accordingly. We are certain that there are no in-vitro experiments or other alternatives possible in order to investigate neurobiological mechanisms of DBS.

The parameters of the Power Calculation are based on our previous study (DEC 2011-040). As readout parameter we make use of the exploration time of the Object Location task. Total exploration time during T1 and T2 (e1 and e2, respectively) is considered as the sum of time spent at both objects. Discrimination performance is calculated as follows (time at object at novel position – time at objects in old position)/e2, in order to correct for possible side biases. At least 7 animals are needed to reach statistical significance. From experience we know that factors such as the complication of surgery and correct electrode localization will lead to an approximately 35% loss of animal per group. For that reason the total number of animals per group has to be adjusted to 9 animals.

We hope that our answers to your questions are more plausible at this instant.

Sincerely,

From: Dec Secretariaat
Sent: woensdag 16 november 2011 9:39
To:
Subject: FW: Dec 2011-137

Attachments: DEC aanvraag formulier (1) (1).doc; DEC brief.doc; Voorblad aanvraag dierproef DEC (1) (1).doc


DEC aanvraag rmulier (


DEC brief.doc (31 KB)


Voorblad raag dierproef I

Geachte onderzoeker, beste

Toch nog even twee opmerkingen van de DEC op je herziene versie die nog aangepast dienen te worden alvorens ik goedkeuring kan geven:

-The sentence under 7c should be removed: "Therefore, at least 10 animals per group will be needed."

-The total nr of animals described under 7c = 32. However, on the frontpage the total nr = 37. This should be in agreement.

Graag je reactie.
Groetjes

-----Original Message-----
From:
Sent: maandag 14 november 2011 16:30
To: Dec Secretariaat
Subject: AW: Dec 2011-137

Dear Mrs.
by accident I have not saved my changes before sending the last email. I am very sorry for this inconvenience and I made sure that this email has the correct files.

Kind regards,

Von: Dec Secretariaat
Gesendet: Montag, 14. November 2011 08:38
An:
Betreff: FW: Dec 2011-137

Geachte onderzoeker, beste

De DEC heeft de herziene bsproken en vindt de vragen nog niet voldoende/cq. goed beantwoord.

Punt 4:
De eerste zin kan verwijderd worden, dit is niet relevant voor de DEC.
De DEC wil hier graag zien staan bijvoorbeeld: Het gebruik van dieren is noodzakelijk aangezien de complexiteit van neurobiologie-welke niet mogelijk te onderzoeken is in vitro (iets in deze trand).

Maastricht, 16-11-2011

Dear

We have adopted your comments in our new DEC with the number 2011-137.

We have deleted the sentence under 7c and adjusted the number of total animals on the front page. We apologize for the inconvenience.

At this instant, everything is in agreement.

Sincerely,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	16-11-2011

Project: *Deep brain stimulation for memory enhancement in an experimental rat model.*

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:	2011-137
Diersoort:	rat
Aantal dieren:	32
Einddatum:	16-11-2015

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

Vicevoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht