Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-091

Ontvangen: 07-07-2011

DEC datum goedkeuring#	Type aanvraag [2]
20-07-2011	Nieuw / ~~Herz.versie / Pilot~~

VROM/GGONR[3]
DGM/SAS IG 07-090

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	30973359E

Titel van het onderzoek:

The role of the serotonergic system in the sensitivity to CO_2 exposure

startdatum September 2011 einddatum [9] September 2013 *Duur van de proef [10]: 2* *mnd ~~/jr~~*

	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]				Art.9	
4. overige uitvoerenden				Art.12	
5.					

Diergroep	1;C	2;C	3;C	4;C	5;C	6;C	7;CO_2	8;CO_2	9;CO_2	10;CO_2	11;CO_2	12;CO_2
ctrl/exp/sham	C	C	C	C	C	C	exp	exp	exp	exp	exp	exp
Diersoort	01	01	01	01	01	01	01	01	01	01	01	01
Stam	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6
Construct / mutatie ?	5-HTT +/+	5-HTT +/+	5-HTT +/-	5-HTT +/-	5-HTT -/-	5-HTT -/-	5-HTT +/+	5-HTT +/+	5-HTT +/-	5-HTT +/-	5-HTT -/-	5-HTT -/-
Herkomst (leverancier) *	01	01	01	01	01	01	01	01	01	01	01	01
Aantal	7	7	7	7	7	7	7	7	7	7	7	7
Geslacht	m	m	m	m	m	m	m	m	m	m	m	m
Dieren immuuncompetent ?	ja	ja	ja	ja	ja	ja	ja	ja	ja	ja	ja	ja
Leeftijd/gewicht	10 weken	10 weken	10 weken	10 weken	10 weken	10 weken	10 weken	10 weken	10 weken	10 weken	10 weken	10 weken
Doel van de proef *	32	32	32	32	32	32	32	32	32	32	32	32
Belang van de proef *	01	01	01	01	01	01	01	01	01	01	01	01
Toxicologisch onderzoek *	01	01	01	01	01	01	01	01	01	01	01	01
Bijzondere technieken *	01	01	01	01	01	01	11	11	11	11	11	11
Anesthesie *	01	04	01	04	01	04	01	04	01	04	01	04
Pijnbestrijding *	01	01	01	01	01	01	01	01	01	01	01	01
Mate ongerief *	03	03	03	03	03	03	04	04	04	04	04	04
Toestand dier einde exp*	01	01	01	01	01	01	01	01	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: The role of the serotonergic system in the sensitivity to CO_2 exposure

1. Doel van de proef.

This project investigates the effects of different concentrations of CO_2 on affective behaviour and neuroanatomical differences depending on the serotonergic genotype.

Panic Disorder (PD) is a severe psychiatric disease with a prevalence of about 3% in the general adult population and is characterized by the occurrence of spontaneous, recurrent and unpredictable panic attacks (PAs), i.e. periods of irrational intense fear and/or discomfort that are accompanied by symptoms such as palpitations, chest pain, breathing difficulties, sweating, nausea and fear of losing control or fear of dying. Experiencing a PA often results in a persistent concern about the recurrence of future attacks, which can lead to a permanent state of anxiety before starting an activity. This, in turn, can have deleterious influences on daily life performance.

Previous research in humans indicated that inhalations of high carbon dioxide (CO_2) concentrations induce fear in both PD patients and healthy subjects. In addition, it has been reported that a dose-dependent fear reaction was moderated by a polymorphism in the serotonin transporter gene (5-HTTLPR). Studies in rodents suggested a role of the acid-sensing ion channel 1a (ASIC1a) in sensing a decreased pH and eliciting fear behavior (Ziemann et al., 2009).

In this novel investigation, we will look at the role of different serotonin transporter (5-HTT) genotypes on the sensitivity to exposure to 0% and 10% CO_2. In particular, we aim to study anxiety- and fear-related behaviour in response to CO_2 exposure depending on the 5-HTT genotype, and we aim to study the underlying neuroanatomical and neurobiological mechanisms, such as levels of ASIC1a.

For this purpose, wildtype (+/+), serotonine transporter (5-HTT) heterozygous (+/-) and homozygous (-/-) knock-out (k.o.) C57BL/6 mice will be subjected to different behavioural tasks, whilst exposed to CO_2. Subsequently, brains will be dissected out and analyzed by means of immunohistochemistry or (epi)genetic analyses. **Heterozygous mice are included for the genotype's clinically relevance, since in human carriers of two s-alleles the 5-HTT expression is diminished, but but not completely absent. Therefore, for specifically studying gene x environment interactions the heterozygous genotype in mice matches this particular human situation best.**

This is a new project, which investigates the interconnection between the 5-HT system, CO_2 evoked fear, and e.g. ASIC1a in a translational manner. A human study with a comparable set-up, i.e. CO_2 exposure and evaluating the modulating role of the 5-HTTLPR, has been performed at the department Psychiatry and Psychology, Maastricht University. By using a similar fundamental approach in animals, crucial additional insights into the pathophysiology of PD might be obtained, which, in turn, would contribute to developing novel treatment strategies.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

About 20% of the general adult population experience at least one panic attack (PA) in their life (Kessler et al., 2006), which is characterized by different sensations including chest pain, palpitations, and breathlessness (American Psychiatric Association, 2000). 3% of the population finally develop panic disorder (PD) consisting of recurrent, unpredictable PA (Weissman et al., 1997). The sensations felt during a PA often result in visiting emergency and/or heart departments without a right diagnosis, which, in turn, reduces the sufferer's quality of life and also results in high health care costs. Further, first-line pharmacological treatment consists of selective serotonin (5-HT) reuptake inhibitors (SSRIs) that target the serotonin transporter (5-HTT) (Ravindran & Stein, 2010). However, efficacy is modest, especially in the long term, and side effects are common

(Cascade, Kalali, & Kennedy, 2009). Therefore, it is essential to gain further insights into the underlying mechanisms of PD and to understand the role of genetic variants in order to provide increased knowledge for developing new therapeutic strategies. Though the proposed experiment has clinical relevance (it is directly linked to ongoing clinical work based on a similar concept), it is fundamental in its nature.

3. Alternatieven

Given that we are studying behaviourally and neuroanatomically related events, including effects on whole body biochemistry, in these animals, alternatives such as cell culture techniques would not be able to suffice. All effort is taken to minimize suffering as much as possible. In addition, a power calculation is used to include the lowest number of animals to obtain statistically relevant data.

4. Ethische afweging

The researchers are convinced that the importance of the planned study outweighs the suffering of the animals involved, given the prevalence of PD, the high health care costs, and absence of long-term effective pharmacological treatment strategies. Gaining knowledge on the molecular mechanisms contributing to the sensitivity to CO_2 in particular and to the development of PD in general will provide important insights for developing improved and/or new treatment strategies.
All effort is taken to minimize suffering of the animals as much as possible.

5. Wetenschappelijke onderbouwing

In the general adult population, about 20% experience at least one panic attack (PA) in their life (Kessler, et al., 2006). A PA is accompanied by sensations such as palpitations, chest pain, breathlessness and dizziness, and avoidance behaviour (American Psychiatric Association, 2000). Some people eventually develop PD, which is characterized by recurrent, unpredictable PA. Prevalence of PD is up to 3% in the general adult population (Weissman, et al., 1997).

Experimentally, PAs can be induced by breathing of an excess concentration of carbon dioxide (CO_2), which has been shown to cause fear in PD patients (Nardi et al., 2006; Perna et al., 2004) and, depending on the used dosage, also in healthy adults (Griez, Colasanti, van Diest, Salamon, & Schruers, 2007). The underlying mechanism has been proposed to rely on changes in brain pH (Esquivel, Schruers, Maddock, Colasanti, & Griez, 2010). In addition, recent data in humans demonstrated a dose-dependent fear reaction to CO_2 that is moderated by a functional polymorphism in the promoter region of the 5-HT transporter (5-HTTLPR) (Schruers et al., 2010). Previous work in rodents showed that homeostatic increases in respiration due to CO_2 administration might be mediated by medullary 5-HT neurons (Richerson, 2004). In addition, recently, the acid-sensing ion channel 1a (ASIC1a) has been shown to be involved in eliciting fear behavior in rodents by detecting decreased extracellular pH (Ziemann, et al., 2009).

Specific Aims

The proposed study aims to investigate how exposure to different concentrations of CO_2, i.e. 0% and 10% CO_2, affects 1) anxiety- and fear-related behaviour, 2) stress-induced corticosterone secretion, 3) the brain 5-HT system (particularly 5-HT and 5-HTT), and 4) central levels of ASIC1a. Moreover, it aims to elucidate whether, and if so, to which extent, these effects are dependent upon the 5-HTT genotype. Understanding the role of proteins involved in 5-HT neurotransmission and/or sensing changes in pH in mediating the sensitivity to CO_2-exposure, will significantly accelerate the development of novel treatment strategies for PD.

For this experiment, at P90 (3 months), both wildtype (+/+), heterozygous (+/-) and homozygous (-/-) 5-HTT k.o. C57BL/6 mice will be subjected to different behavioural tasks, whilst exposed to either 0% or 10% CO_2 (depending on the experimental group). In addition, stress-induced corticosterone levels will be measured. For a more detailed description, see sections 7 and 8, and the SOPs in Appendix 1.

6. Wetenschappelijke beoordeling

The present DEC protocol has been examined and approved by , ( ), and , Associate Professor Maastricht University, who verified that the translational component is adequately incorporated. Principal investigator is , who is experienced with rodent experiments and the used mouse strain.

7. **Proefdier keuze**

7a. Soort, stam / herkomst / eindbestemming

Wildtype (+/+), heterozygous (+/-) and homozygous (-/-) 5-HTT k.o. mice (background C57BL/6) will be used to evaluate the role of the 5-HT system on the sensitivity to CO_2 exposure. The mice will be obtained by in house breeding (the required breeding pairs will be obtained from the lab of Prof. Lesch in Wuerzburg, Germany).

7b. Sexe

Only male animals will be used in this experiment, since it has been reported that the response to CO_2 exposure depends on the estrous cycle (Olsson et al., 2003). Therefore, to reduce variation, only male offspring will be used.

7.c. Aantallen

For behavioural testing this investigation requires a total of 84 mice, divided over six experimental groups (n = 14/group, 6 x 14 = 84)*:

1) 5-HTT +/+ 0% CO_2
2) 5-HTT +/- 0% CO_2
3) 5-HTT -/- 0% CO_2
4) 5-HTT +/+ 10% CO_2
5) 5-HTT +/- 10% CO_2
6) 5-HTT -/- 10% CO_2

*+/+: wildtype, +/-: heterozygous, -/-: homozygous

Half of each group (n = 7/group) will be used for immunohistochemical analysis and the other half per group (n = 7/group) for (epi)genetic analyses.

The required number per group is based on a power calculation using the formula of Sachs with the following parameters: alpha = 0.05, power = 0.8, sigma = 0.22, delta = 0.25, m = 1 (based on the elevated zero maze experiment). From this calculation, a sample size of 12 per group is derived. However, based on experience with mice we expect that about 15% (n = 2) of the animals will show insufficient exploratory behaviour. Therefore, we plan to include 14 animals per group. We do not expect any further drop-out (0%).

8. Experiment

The experiments will be performed with wildtype (+/+), heterozygous (+/-) and homozygous (-/-) 5-HTT male mice that were bred in the CPV (original breeding pairs derived from).

Upon admission to the experiment (approximate age: 10 weeks / P70), mice will be housed individually within a temperature-controlled environment (21±1°C) with a reversed 12hr light/12hr dark cycle (lights on from 7 p.m. to 7 a.m.) and access to standard mouse chow and water ad libitum.

From P90 (3 months) onwards, mice will be subjected to anxiety- and fear-related behavioural tasks (whilst exposed to 0 or 10% CO_2, depending on the experimental group), i.e. a CO_2 aversion assay and the open field test including CO_2 evoked freezing and the elevated zero maze. In addition, stress-induced corticosterone secretion will be examined. At P120 (4 months), when behavioural experiments are completed, animals will be sacrificed, brains will be removed from the skull and processed for further analysis. Brains of half of the animals per group (n = 7/group) will be utilized for immunohistochemical analysis (animals sacrificed by transcardial perfusion), the other half per group (n = 7/group) will be processed for (epi)genetic analyses using micro-array (animals sacrificed by decapitation).

Experimental procedures:

Experimental groups:

Group number	Condition	Genotype*	Number	Stress level (VHI)
1	0% CO_2	5-HTT +/+	14	3
2		5-HTT +/-	14	3
3		5-HTT -/-	14	3
4	10% CO_2	5-HTT +/+	14	4
5		5-HTT +/-	14	4
6		5-HTT -/-	14	4

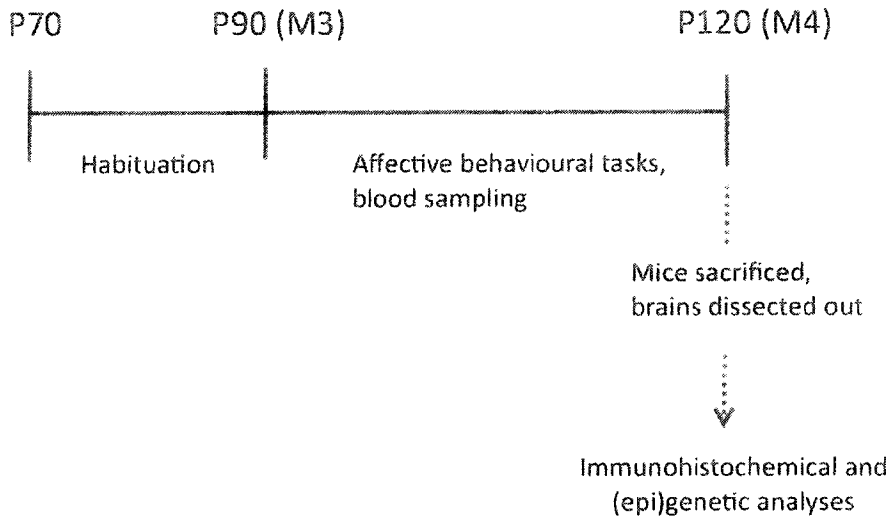
*5-HTT: serotonine transporter, +/+: wildtype, +/-: heterozygous, -/-: homozygous k.o.

Historical control groups do not exist.

All groups:

- Mice will be weighted weekly
- Anxiety- and fear-related behaviour testing (CO_2 aversion assay, open field test with CO_2 exposure including CO_2 evoked freezing, elevated zero maze with CO_2) will be done from P90 onwards, once for each animal
- Stress-induced plasma corticosterone secretion will be evaluated after the behavioural analysis via saphenous vein puncture
- At P120 half of the animals per group (n = 7/group) will be anesthetized using pentobarbital (100 mg/kg, i.p.) and intracardially perfused using Somogyi fixative (4% depolymerized paraformaldehyde, 0.05% glutaraldehyde, 15% picric acid in 0.1 M phosphate buffer (pH 7.4)), after which brains will be removed quickly and processed for further examination by immunohistochemistry. The other half of the animals (n = 7/group) will be sacrificed using decapitation, followed by dissection of the brain and processing for future (epi)genetic analyses.

Experimental timeline:



Abbreviations: P: postnatal day, M: month

9. Experimentele condities

9a. Anesthesie

After completion of all behavioural testing, half of the mice per group (n = 7/group) will be deeply anesthetized using pentobarbital (100 mg/kg, i.p.) and euthanized by intracardial perfusion using Somogyi fixative. The other half of the animals (n = 7/group) will be quickly decapitated without prior stretching. After sacrificing the animals, brains will be dissected out.

No other anesthetics are given since the stress involved is known to influence the gene expression and epigenetic modifications in the brain (Hunter, McCarthy, Milne, Pfaff, & McEwen, 2009), parameters we aim to study .

9b. Pijnbestrijding

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

9c. Euthanasie en Humane eindpunten

At P120, after behavioural testing, half of the mice per group (n = 7/group) will be deeply anesthetized with pentobarbital (100 mg/kg, i.p.) and subsequently intracardially perfused with Somogyi fixative consisting of 4% depolymerized paraformaldehyde, 0.05% glutaraldehyde and 15% picric acid in 0.1M phosphate buffer (pH 7.4). Brains will be removed and utilized for further examination by means of immunohistochemistry. The other half of animals per group (n = 7/group) will be sacrificed by decapitation without prior sedation, since the stress involved is known to have an impact on the brain's gene expression and related epigenetic processes.

"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 decapitation.

Zorg

10a. Ongerief

Mice will be housed individually because it has been observed that the 5-HTT influences aggressive behaviour (Holmes, Murphy, & Crawley, 2003). To avoid confounding behaviour animals are housed solitary. **For discomfort scores per group see the following table:**

Group	*Exposure*	*Duration*	*Frequency*	*Stress Level*
1	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	03
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	0	-
	Euthanasia	n.a.	1	03
2	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	03
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	1	03
	Euthanasia	n.a.	1	03
3	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	03
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	0	-
	Euthanasia	n.a.	1	03
4	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	03
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	1	03
	Euthanasia	n.a.	1	03
5	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	03
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	0	-
	Euthanasia	n.a.	1	03
6	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	03
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	1	03
	Euthanasia	n.a.	1	03
7	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	04
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	0	-
	Euthanasia	n.a.	1	03
8	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	04
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	1	03
	Euthanasia	n.a.	1	03
	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	04
	~~Blood sampling stress~~	~~2 hours~~	~~1 session"~~	~~03~~

9	responsivity			
	Injection (i.p.)	n.a.	0	-
	Euthanasia	n.a.	1	03
10	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	04
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	1	03
	Euthanasia	n.a.	1	03
11	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	04
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	0	-
	Euthanasia	n.a.	1	03
12	Solitary housing	3 months	n.a.	03
	Affective behavioral tasks	4 weeks*	1 (each task)	04
	Blood sampling stress responsivity	2 hours	1 session"	03
	Injection (i.p.)	n.a.	1	03
	Euthanasia	n.a.	1	03

*every individual animal is tested only 3 times for maximally 20 minutes during that period
"1 session consists of 3 sampling moments

The total discomfort score for animals not exposed to CO_2 (groups with odd numbers, 1st column) is 03 and score 04 for animals exposed to CO_2 (groups with even numbers, 1st column).

10b. Welzijnsevaluatie
Will be taken for. Relevant data from previous investigations: n.a.

11. Verzorging en huisvesting
Mice will be housed at level 00 (CPV). Behavioural experiments, blood collection and i.p. injection will be performed at level 00 (CPV). The CPV will be responsible for changing cages, food and water supply. In case of problems, please contact the

12. Deskundigheid
The P.I. (art. 9 WOD) has 9 years experience with animal models in general and also previously worked with 5-HTT k.o. mice. will assist in executing the project. No non-authorized foreign employees or students are involved in this investigation.

13. Standard Operation Procedures (SOP)
See Appendix 1.

Relevante literatuur

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Appendix 1: SOPs

Affective behavioural tasks

Open Field Test (OFT) and CO_2-evoked freezing

Animals are tested on the OFT as previously described (Brummelte, Pawluski, & Galea, 2006; Van den Hove et al., 2005). This test is conducted in a square Plexiglas base (50 x 50 cm) with a white floor and 40-cm high transparent Plexiglass walls. The arena is subdivided into a 30 x 30 cm central zone, 10 x 10 cm corners and 30 x 10 cm walls. Immediately after the mouse is placed in the centre of the open field, the movements of the mouse are scored automatically with a computerized system (Ethovision Color Pro, Noldus, The Netherlands). The floor of the open field is cleaned with ethanol and dried thoroughly after each session. Testing is carried out for a 20 min period, once per animal. The time spent in the different zones and total distance moved are scored by the automated-tracking system. An observer blind to the conditions and genotype scores the number of rears and the number of fecal boli from each animal. An increased number of central crossings is considered indicative of decreased anxiety-like behaviour, while increased total crossing is considered as index of locomoter behaviour (Prut & Belzung, 2003). In addition, CO_2-evoked freezing is scored.
CO_2 is administered via tubes after placing the animal in the centre of the open field. Within a short period of time a steady state is reached.

CO_2-Aversion Assay

Mice are placed in a transparent Plexiglas chamber consisting of two parts (each 25 x 25 cm with 40 cm high walls), one will be filled with 10% CO_2 and one with air, connected by a swinging door. Mice are allowed to move and cross freely between the two chambers. Time spent in each side is scored. The side of CO_2 administration is randomly. Movements and chamber crossings are videotaped for a period of 10 min and evaluated by an observer blind to experimental condition and genotype. Prior to testing, mice are put into the chamber without CO_2 exposure to learn crossing between the two chambers for at least four times as previously described (Ziemann, et al., 2009).

Elevated Zero Maze (EZM)

The zero-maze, described in Shepherd et al. (1994), consists of a circular alley (diameter of 25 cm). The path has a width of 5 cm. The maze is divided in four parts, i.e., two opposite open parts and two opposite closed parts. The sidewalls have a height of 25 cm. The open parts have borders with a height of 5 mm. The parts of the maze, which the animals could have contact with, are made from a black plastic material that was transparent for infrared light. The maze is elevated 20 cm above the floor. The light intensity in the open arms is ± 2 lux, in the closed arms ± 1 lux. The maze is placed under a metal frame with black curtains on every side and will be performed in a closed box filled with CO_2.
For the test, the mouse is placed into one of the open parts facing the closed part of the apparatus for five minutes. After the test the mouse is removed from the apparatus and the maze was cleaned with 10 % ethanol and dried thoroughly. The route followed by the mouse is tracked by a video camera connected to special equipment (Ethovision Pro, Noldus, The Netherlands). The software is capable of measuring the number of visits, the distance travelled and the time spend in each part of the maze.

Stress responsivity

To investigate responsivity to stress, animals are placed in a small (mouse) cage filled with 250 ml water for 20 min. Blood samples are collected from saphenous vein puncture (see

below) prior to placement in the apparaturs (within 3 min of entering the experimental room), 20 min after placement in the apparatus, and 60 min after the onset of the stress.

Saphenous vein puncture
CPV SOP #: CPV-3_MR
Goal: Repetitive blood sampling (50 µl/time) via de saphenous vein.

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

Preparations
- The 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://ww.uib.nl/vivariet/mou_blood/Blood_coll_mice_.html

Perfusions
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
CO_2-evoked freezing, CO_2 aversion assay, Open field test (non-escapable open field stress), Elevated zero maze:

in presence of CO_2 and evoking of panic: code 04

in presence of CO_2 and without evoking panic or in absence of CO_2: code 03

Saphenous vein puncture: code 03

Perfusion: code 03

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Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol, 463*(1-3), 3-33.

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Ziemann, A. E., Allen, J. E., Dahdaleh, N. S., Drebot, II, Coryell, M. W., Wunsch, A. M., et al. (2009). The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. *Cell, 139*(5), 1012-1021.

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, 29-06-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*The role of the serotonergic system in the sensitivity to CO_2 exposure*", is op de DEC vergadering van 24 juni 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt de kolommen op het voorblad op te splitsen, je kunt niet 2 coderingen in een kolom gebruiken (anesthesie).
- De DEC wenst een onderbouwing bij punt 1, van de heterozygous (+/-) groep.
- Bij punt 6 verzoekt te vermelden dat dit "**DEC**" protocol has been examined and approved by
- Bij punt 7a verzoekt de DEC de laatste alinea bij punt 9a te vermelden. De DEC prefereert ogenblikkelijk decapitatie en niet eerst strekken.
- Bij punt 7c verzoekt de DEC de uitval in percentages uit te drukken en verzoekt niet tussentijds af te ronden (de uitval van het afgeronde percentage berekenen).
- Bij punt 8 (all groups) vraagt de DEC zich af of het wel of niet blootstellen aan CO_2, de uitkomst van de CO_2 gebaseerde testen beïnvloedt.
- Bij punt 9a wenst de DEC een onderbouwing waarom er geen verdoving wordt gegeven.
- Bij punt 10a verzoekt de DEC in een tabel, de aard, ernst, duur en frequentie beter te definiëren per handeling en groep, en het totale ongerief aan te geven (ook de wijze van euthanasie dient in het ongerief meegenomen te worden).

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 2011-091, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

/Voorzitter DEC-UM

Betreft: DEC-aanvraag 2011-091

Maastricht, 05-07-2011

Geachte DEC,

Hieronder vindt u onze respons op de verscheidene vragen en opmerkingen van de DEC aangaande onze projectaanvraag: "*Low-grade inflammation and the pathogenesis of major depression: identification of molecular markers*".

Hopende u zo voldoende te hebben geïnformeerd, groet ik,

- De DEC verzoekt de kolommen op het voorblad op te splitsen, je kunt niet 2 coderingen in een kolom gebruiken (anesthesie).
  Is aangepast; zie voorblad.

- De DEC wenst een onderbouwing bij punt 1, van de heterozygous (+/-) groep.
  De +/- groep is klinisch gezien nog het meest relevant; zie verdere onderbouwing in DEC aanvraag.

- Bij punt 6 verzoekt te vermelden dat dit "DEC" protocol has been examined and approved by

  Is aangepast; zie DEC aanvraag.

- Bij punt 7a verzoekt de DEC de laatste alinea bij punt 9a te vermelden. De DEC prefereert ogenblikkelijk decapitatie en niet eerst strekken.
  Is aangepast; zie DEC aanvraag.

- Bij punt 7c verzoekt de DEC de uitval in percentages uit te drukken en verzoekt niet tussentijds af te ronden (de uitval van het afgeronde percentage berekenen).
  Is aangepast; zie DEC aanvraag.

- Bij punt 8 (all groups) vraagt de DEC zich af of het wel of niet blootstellen aan CO2, de uitkomst van de CO2 gebaseerde testen beïnvloedt.
  Dit is juist wat dit project beoogd met dit experimentele design. Blootstelling aan 10% CO2 leidt tot een verhoogd angst-gerelateerd gedrag in muizen (zie referentie Ziemann, DEC aanvraag). Wij willen daarbij onderzoeken of het serotonerge system hierbij een rol speelt, dus of muizen met verschillende serotonerge genotypes een verschillende respons op CO2 laten zien.

- Bij punt 9a wenst de DEC een onderbouwing waarom er geen verdoving wordt gegeven.
  Is aangepast; zie DEC aanvraag.

- Bij punt 10a verzoekt de DEC in een tabel, de aard, ernst, duur en frequentie beter te definiëren per handeling en groep, en het totale ongerief aan te geven (ook de wijze van euthanasie dient in het ongerief meegenomen te worden).
  Is aangepast; zie DEC aanvraag.

From:
Sent: dinsdag 19 juli 2011 12:49
To: Dec Secretariaat
Cc:
Subject: RE: Project 2011-091-w
Attachments: DEC2011-091 _3 (3).docx

Geachte DEC, beste

- Wat betreft het eerste punt, is het zo dat de controlegroepen nooit aan CO2 worden blootgesteld, waardoor er in deze groepen dus geen additioneel angst-gerelateerd gedrag wordt uitgelokt. Blootstelling aan 10% CO2 is alléén van toepassing op de CO2 groepen en dan ook nog eens alléén tijdens degedragstaken zelf (hetgeen naar verwachting leidt tot een verhoogd angst-gerelateerd gedrag in muizen; zie referentie Ziemann, DEC aanvraag). De rest van de vraag is derhalve niet meer van toepassing.
- Met betrekking tot het tweede punt, heeft eerder onderzoek aangetoond dat acute stress (in ons geval een injectie) een veranderde methylatie (epigenetische process) in het brein teweeg brengt (Hunter et al., 2009). Daar we juist dit soort epigenetische processen willen bestuderen, willen we additionele stress bij euthanasie vermijden.

Zie ook de bijgevoegde DEC-aanvraag.
Met vriendelijke groet,

From: Dec Secretariaat (
Sent: woensdag 13 juli 2011 12:16
To:
Subject: FW: Project 2011-091-w

Geachte Onderzoeker, beste

De DEC heeft je herziene versie besproken en heeft nog de volgende vragen/opmerkingen:

- De DEC heeft begrepen wat met het project beoogd werd. Het punt is dat uit de protocollen geinterpreteerd kan worden dat het angst-gerelateerde gedrag wordt uitgelokt met een CO2 stimulus (in zowel de controle als de chronisch CO2 blootgestelde groep). Als dit correct is, is de vraag van de DEC of de continue CO2 blootstelling zou kunnen interfereren met het angst-gerelateerde gedrag door (weer) CO2 stimulus. In dat geval is de controle groep niet vergelijkbaar met de (chronisch) CO2 blootgestelde groep en lijkt een andere stimules om angst-gerelateerd gedrag uit te lokken aan te bevelen

- Punt 9a- De DEC verzoekt om concreet bewijs (referentie, eerdere data), dat verdoving inderdaad interfereert met de te meten parameters.

Graag je reactie.

Met vriendelijke groet namens DEC-UM

Ambtelijk Secretaris Dierexperimentencommissie

Postbus 616-UNS 50-Box 48, 6200 MD Maastricht

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	22-07-2011

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht

Project: *The role of the serotonergic system in the sensitivity to CO_2 exposure.*

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:	2011-091
Diersoort:	muis
Aantal dieren:	84
Einddatum:	20-07-2015

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