PROMETEUS

preterm brain-oxygenation and metabolic eu-sensing

D3.1 - Ethics rats

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PROMETEUS
preterm brain-oxygenation
and metabolic eu-sensing

Grant Agreement	101099093
Acronym	Prometeus
Project full title	Preterm Brain-Oxygenation and Metabolic EU-Sensing: Feed the Brain

Deliverable	**D3.1**
Deliverable Name	**Ehics rats**
Nature of deliverable	ETHICS: Deliverables related to ethics issues.
Dissemination level	PU (Public – fully open automatically posted online on the Project Results platforms)
Scheduled delivery date	31/05/2023
Actual delivery date	17/10/2023

Prepared by	Dr Emmanuel Barbier
Reviewed by	
Verified by	Dr Marta Pozza

History of Changes

Revision	Date	Author	Changes	Status
v. 1	05/10/2023	Emmanuel Barbier		Final

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1. Objective to conduct studies in animals

The aim is to provide a framework for personalized brain nutrition in premature babies, based on real-time monitoring of cerebral blood flow and oxygenation on the one hand, and blood metabolites on the other. Current knowledge is limited to adults. To achieve this goal, we will study the relationship between different key brain energy fuels, cerebral blood flow and cerebral oxygenation on the basis of data obtained in raccoons under different physiological conditions.

2. Application of the 3R rule

Replacement	No in vitro preparation can reproduce the relationship between the brain's various key energy fuels (oxygenation, blood flow), including its response to diet and blood glucose (oxygenation, blood flow), including its response to diet and blood sugar.
Reduction	The number of rats required for our work was reduced to a minimum without compromising the statistical interpretation of our results. In order to quantify our numbers and obtain a sufficient number of interpretable data, we have taken into account the technical problems that may arise during the creation of the animal model on the one hand, and during medical medical imaging on the other hand.
Refinement	A week of acclimatization is planned for the arrival of the animals before any experience. The animals are housed in enriched cages, according to the protocol in place at the animal housing facility. The health status of rats and pups will be monitored throughout the experiment. Before birth, paper-based nesting material is placed in the cage. For pregnant and lactating rats, we will monitor the following criteria: prostration, sunken eyes, cessation of fur cleaning. This will enable us to intervene immediately and appropriate action at the slightest sign of suffering (in particular, pain relief through the painkillers). For pups, during the suckling phase (from birth to two weeks after birth), we will monitor birth), we monitor any rejection of the nest by the mother. For pups after separation separation from their mother, the litter will be kept (all the animals together) as well as the nesting material, impregnated with the mother's odour. The nesting material will help regulate the animals' temperature, and the impregnated nesting material impregnated with the mother's odour will limit stress. Humidified food is placed in the cage in the cage, along with a full, dripping water bottle to make it easier for the pups to find the water source. The pup's belly, when it's still transparent enough, will be monitored to ensure that the pup are feeding properly. The pup's belly will be massaged twice a day to favour defecation. For all blood analysis and imaging experiments, the animals will be anaesthetized and will receive analgesics. An adapted scoring grid will be used to identify appropriate early limit points, and to define criteria for stopping suffering.

2.1 Number of animals

Mother rat: 171 (will be reused for other experiments)
Rat pups: 2018 (will undergo an anaesthesia without awakening)

2.2 Impact of animals

For rat pups: artificial nutrition of pups separated from their mothers before complete weaning for 3 days, followed by a 3-hour terminal procedure including anesthesia, blood analysis, medical

imaging, hypoglycemia or hyperglycemia, euthanasia. Level of suffering: moderate

For female rats: 1 euthanasia after four litters. Level of suffering: mild

2.3 Application process

The ethical application was drafted between June and September 2023. Forms were submitted to the Ethical Committee of the Grenoble Institut Neuroscience. A version was then submitted to the French Ministry of Research, revisions were requested, corrections were made and the ethical agreement was finally granted on the 5th of October 2023.
The ethical agreement is valid 5 years from the day of its approval.

2.4 Authorization

MINISTÈRE
DE L'ENSEIGNEMENT
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ET DE LA RECHERCHE
Liberté
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Service de la performance, du financement et de la contractualisation avec les organismes de recherche

Département des pratiques de recherche réglementées

Cellule Animaux utilisés à des Fins Scientifiques - AFiS

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Direction générale
de la recherche et de l'innovation

Paris, le 5 octobre 2023

Autorisation de projet utilisant des animaux à des fins scientifiques

En application des dispositions du code rural et de la pêche maritime, notamment ses articles R.214-87 à R.214-126, le projet :

1. référencé sous le numéro APAFIS #[redacted]
2. ayant pour titre : [redacted]
3. déposé par l'établissement utilisateur : Institut des Neurosciences de Grenoble (GIN), Inserm U1216, numéro d'agrément C3851610008, dont le responsable est Monsieur Emmanuel BARBIER
4. et dont la responsabilité de la mise en œuvre générale et de la conformité à l'autorisation est assurée par : [redacted]

est autorisé.

L'autorisation de projet est accordée, sous réserve de la validité de l'agrément de l'établissement, pour une durée de 5 ans à compter de la présente notification.

Le projet précité a été évalué sur le plan éthique par le comité d'éthique en expérimentation animale n°004 et a reçu un avis favorable.

Ce projet n'est pas soumis à l'obligation d'une appréciation rétrospective à l'issue de sa réalisation.

Pour la ministre et par délégation
le chef du département des pratiques
de recherche réglementées

Laurent PINON