1 Publishable summary

Brain diseases are one of the most prevalent groups of diseases in Europe with estimated annual costs amounting to 386 billion Euros. Data collected by the WHO suggest that brain diseases are responsible for 35% of Europe's total disease burden. In the treatment of neurological disease, the blood brain barriers (BBB) still represent an obstacle for the delivery of drugs to the brain and thus a major challenge for the development of therapeutic regimens. Understanding the molecular basis and functioning of the BBB in health and disease, including transport mechanisms across the BBB, therefore holds significant potential for future strategies to prevent and ameliorate neurological disease. Recent research indicates that the cause of some neurological disorders can be traced to insults during early developmental stages. The major goal of the NEUROBID project is thus to understand the molecular mechanisms and function of the BBB in health and disease, both in the developing brain and the adult central nervous system. The interdisciplinary consortium from the fields of developmental neurobiology and BBB research will seek to (i) understand the involvement of normal and disturbed BBB function in normal and abnormal brain development and (ii) to develop novel strategies for drug delivery to the brain. Unique transport mechanisms across the BBB will be used to target potential therapeutic macromolecular and cellular agents specifically to the brain barriers and transport them into the brain. NEUROBID will focus primarily on non-inherited neurodevelopmental disorders arising from perinatal adverse exposure, such as cerebral palsy, and classic adult neurological disorders, such as multiple sclerosis and stroke. In the long term, NEUROBID hopes to pave the way for new treatment strategies and thus reduce the economic and social burden of neurological disease.

Scientific Achievements

WP1 – Normal development

The NEUROBID project will specifically allow significant advances in:

i) establishing the developmental profile of the molecular components essential to the blood-brain barrier integrity and transport functions

ii) elucidating the molecular basis for large molecule (protein) transfer across the choroid plexus

iii) identifying specific brain barrier cell surface recognition targets

This will have advanced to a stage where realistic comparisons can be made with the molecular and functional status of these barrier mechanisms in the pathophysiological animal models to be studied in WP2 and WP3 and in the barrier protection studies in WP4. This will provide better understanding of the pathological mechanisms in these neurological conditions and lead to development of new brain barrier protectants (WP4) and new treatments achieving an increased and specific delivery to the brain (WP5).

In the 3rd Reporting Period considerable work has been done on the completion of the report on the outcome of the molecular and morphological studies designed to define cell surface and intracellular large molecule influx transporters at brain barrier interfaces (blood-brain and blood-CSF) and their main efflux transporters in the developing brain.

WP2 – Adverse perinatal exposures

The functions fulfilled by the BBB are challenged by adverse perinatal exposures such as inflammation, hypoxia and glucocorticoid treatment. There is only very limited knowledge on the degree and molecular mechanisms of alteration of these functions in the developing BBB during these pathological states.

Obtaining this information is important for two reasons:

1. It is required to devise and optimize strategies to protect the BBB during adverse perinatal exposures and thereby prevent brain damage during development and protect against the long term effects of perinatal BBB defects for defects, which may lead to adult neurological and psychiatric disease.

2. Detailed knowledge on BBB activation in the context of adverse events is required to optimize pharmacotherapeutic intervention with large molecules as the information obtained can be used to target large molecules across the BBB. WP2 will be the first comprehensive study of the effect of disease on BBB function in the developing individual and will also provide a basis for protection and bypassing of the BBB with the aims of disease prevention and treatment (WP4, 5).

We will use four classical, well characterized and complementary models of adverse perinatal exposure and assess their impact on the integrity of the BBB (Partners 2, 3, 4, 7, and 8). A fifth model, cuprizone demyelination, will be used as a model of early demyelination (Partner 1):

1. Systemic inflammation: administration of the TLR4 agonist LPS (Partners 4 and 7), the TLR3 agonist poly I:C (Partner 4) or the cytokine IL-1 (Partner 2).

2. Hypoxic-ischemic brain damage: unilateral carotid artery occlusion followed by hypoxia (Vannucci model) or injection of glutamate analogues in the neocortex (excitotoxic model) at various stages of development, from day 0-3 after birth until adulthood (Partners 3 and 7)

3. Double hit hypothesis (inflammation followed by hypoxic-ischemic or excitotoxic insult): Administration of LPS, the TLR3 agonist poly-IC, or the cytokine IL-1-beta prior to the Vannucci or excitotoxic model (Partners 2, 4, and 7).

4. Corticosteroids: are among the most powerful drugs used in the perinatal and neonatal period but adverse side effects including neurological impairment, reduced fetal growth, increased risk of neurodevelopmental disability, and cerebral palsy have been reported. Moreover, fetal corticosteroid exposure may sensitize for adult degenerative disease and a role for the BBB has been postulated in all these adverse effects. Partner 8 will use a mouse model of antenatal and neonatal corticosteroid treatment.

5. Examination of the cuprizone effects in the neonatal period (Partner 1). Cuprizone is used as a model of demyelination in the adult brain (see WP3) and appears not to affect the BBB; but its effects in early brain development are unknown and may provide insights into the effects of disruption of pre-myelination stages of brain development (in the absence of a BBB effect) by interference with immature oligodendrocytes or other pre-myelination events. These studies also provide essential background for WP3.

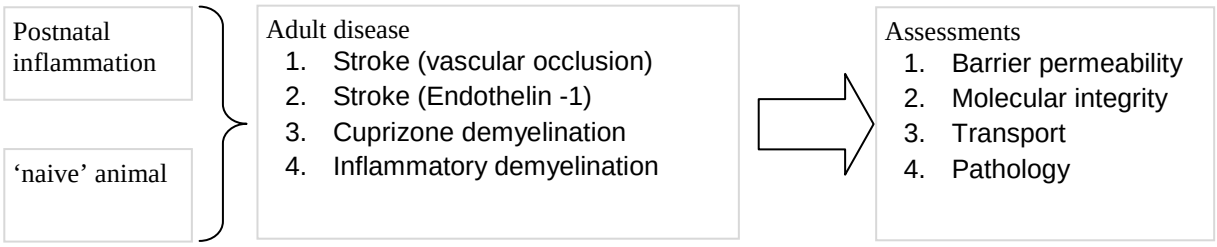
In the 3rd Reporting Period work has focused on the report on consequences of adverse perinatal exposures including inflammation on the function of the brain barrier and on expression of endothelial activation determinants.

WP3 – Adult injury and disease

BBB dysfunction occurs in most principal neuropathologies, but the molecular changes responsible for altered BBB integrity in these diseases are unclear. The immediate impact of adverse perinatal events on the BBB are studied in WP2. WP3 will focus on the long term effects of inflammation on the BBB in healthy adults and in adults with neuropathology. The changes to the BBB, in terms of molecular structure and transport, are likely to be different in adult disease following perinatal inflammation as compared to inflammation in naïve individuals, and would account for the increased brain damage. A better

understanding of these processes is required to develop and optimize strategies to protect the BBB (WP4) and to improve delivery of large molecules across the BBB (WP5).

Disease models



Systemic inflammation will be induced by intraperitoneal injections of lipopolysaccharide (LPS) or interleukin-1beta (IL-1β) in early development and its consequences for the BBB in adulthood will be determined immediately before and after the second insult in adulthood.

The models of adult neuropathology to be used are stroke and demyelinating disease. In both cases we are using two models, one where BBB dysfunction is known to occur and one where the BBB is thought not to be involved. Comparison of these models, will allow us to determine the importance of barrier disruption in disease pathology and the long-term consequence of perinatal inflammation on adult BBB functioning and neuropathology. These complimentary models of perinatal inflammatory insult (Partners 2, 4 and 7) and adult neuropathology (Partners 1, 4, 7, 8) are already well-established within the consortium.

In the 3rd Reporting Period, the focus of work has been on two tasks:

- Report on the contribution of the blood-brain barrier and an early inflammatory response in adult brain neuropathology
- Report on BBB function in adult disease with and without perinatal inflammatory response and the effect on disease severity with and without aspects of barrier involvement

WP4 – Protection and restoration

Oxidative stress and inflammation are believed to be key contributors to the pathogenesis of both BBB breakdown and neuropathology, however, there is little information on how drugs that interfere with these processes protect and/or restore the barrier. In this work package we will use both *in vivo* and *in vitro* models as described in WP1 and WP2 to investigate specific novel protective targets and available neuroprotective drugs focusing on the following:

1. Toll-like receptors (TLRs), which have been identified as key targets for inflammatory processes. Exposure to TLR agonists during development results in reduced myelination in the brain and increased vulnerability to hypoxia-ischemia. However, their role in BBB disruption is unknown.
2. Anti-oxidants and anti-inflammatory drugs have neuroprotective effects against excitotoxic and hypoxic-ischemic brain damage. These neuroprotective agents are likely to act, at least partially, through restoring/maintaining the integrity of the BBB.

In the 3rd Reporting Period the consortium has worked mainly on the report on integrity of BBB and neuroprotection after treatment with anti-inflammatory and anti-oxidants drugs and genetic deletion of novel inflammatory targets following LPS and/or HI.

WP5 – Translation

Normally transfer of macromolecules or cells from blood to brain is blocked or selectively facilitated. In order to bring large therapeutic compounds into the brain the compounds need to remain stable in blood and cross the BBB in sufficient amounts. Methods previously attempted to achieve this have involved modifications of the molecules to increase their lipid solubility, use of inward transporters, endocytotic pathways or encapsulated vectors such as liposomes, nanoparticles, or viral vectors. However, none of these methods are targeted to the brain and thus the concentration of the compound at the BBB interface may be too low due to dilution by whole body distribution and to widespread non-specific entry into non-target cells elsewhere. To overcome this problem several strategies will be used:

1. In WPs 1, 2,and 3 we will identify cell surface structures specific for the (activated) BBB in normal development and disease.These BBB-specific cell surface structures will be targeted using antibodies or specific ligands to discriminate barrier from non-barrier cells and thus optimize specific delivery in the brain. These antibodies or specific ligands will be:

- attached to vehicles (nanoparticles or liposomes) carrying large molecules or
- overexpressed on the surface of stem cells molecularly engineered to produce these large molecules.

2. Utilisation of the transcellular protein transfer mechanisms in developing and adult choroid plexus (to be studied in WP1). This mechanism has the potential for delivery of large molecules (proteins) or other compounds attached to the proteins.
3. Building onto existing *in-silico* physiology-based pharmacokinetic models to predict the delivery and concentration of both small and large molecules to the CNS (and out of the CNS)
4. Translation of basic scientific strategies or technical novelties to clinicians is often hampered by the lack of clinical/scientific networks. Therefore, the establishment of such networks encompassing science groups and clinical groups is an additional goal of this WP.

In the 3rd Reporting Period the following tasks have been fulfilled:

- Targeted delivery of large molecules via nanoparticles, stem cells, liposomes or cationic peptides across the BBB throughout development and in our disease models
- Initiation of clinical scientific network design

The results obtained from the two tasks have been recorded in deliverable 5.1 Report on uptake of carrier-bound large molecules into the brain by targeting them to specific BBB surface markers.

WP6 – Exploitation and dissemination

Strategies for the dissemination and exploitation of the project results and for the protection of intellectual property rights (IPR) have been extended. The website is continuously being maintained and updated to stimulate interactive exchange of information between the interested public and stakeholders. All IPR-related aspects were regulated in the consortium agreement which was signed before the start of the project. The plan for use and dissemination of the foreground has been updated at month 48. Both dissemination and exploitation are pivotal in the NEUROBID project. In support of the above project aims, the NEUROBID consortium is committed to a broad and targeted dissemination of the knowledge generated in order to i) promote a better understanding of the BBB and its functioning, particularly in the developing brain; ii) take first steps to translate this knowledge for future use in clinical intervention and

thus iii) contribute to the basis needed to improve the management of neurologic diseases and ultimately reduce their burden. As such, the key stakeholders targeted by the project are the scientific community, in particular in the field of neurodevelopmental brain research, biomedical/pharmaceutical industry and SMEs, clinicians and other health professionals, and the general public. The central dissemination activities for the scientific and research community are joint scientific publications in high-impact journals and the presentations of project results at high-level conferences and in workshops to be held in the course of the project.

WP7 – Management

In WP 7 the management team maintained the earlier established effective lines of communication and reporting procedures to ensure the adequate planning, implementation and coordination of project activities and an independent continuous assessment of progress for the entire project duration. The management team, assisted by an administrative manager, ensured proper financial management within the consortium and the appropriate communication of related matters to the European Commission. A continuous effort is undertaken by the management team to ensure the timely submission of deliverables, milestones, financial statements and reports.

Continuous monitoring of the project progress is a second focus of the management team. This is ensured through implementation of biannual progress reports and monitoring of deliverables and milestones in the project through the Administrative Manager.

Expected impact

The major immediate impact of the NEUROBID project is its contribution towards a better understanding of the normal characteristics and function of the developing BBB. It will also lead to improved knowledge about its dysfunction, protection and restoration in disease. As a long-term impact, we are confident that the NEUROBID project has the strong potential to make a substantial contribution towards the reduction of neurologic disease burden in children and adults. We believe that the translational results of NEUROBID will lead to improved management strategies for neurologic disorders with the potential to reduce the associated high healthcare costs.

The potential applications that might arise from this project involve novel strategies for drug delivery to the brain. A better understanding of the involvement of normal and disturbed BBB function in normal and abnormal brain development and this translation of this knowledge to potential new clinical and industrial development of mechanisms and interventions also holds the potential to strengthen European leadership in clinical therapeutics.

List of partners

Beneficiary No.	Beneficiary name	Short name	Country
1(CO)	Medizinische Hochschule Hannover THP: Leibniz Universität Hannover	MHH LUH	Germany
2	Institut National de la Santé et de la Recherche Médicale	INSERM : 2L (Lyon) and 2P (Paris)	France
3	Universitair Medisch Centrum Utrecht	UMC Utrecht	The Netherlands
4	The University of Oxford	UOXF.AV	United Kingdom
5	Simcyp Limited	Simcyp	United Kingdom
6	University of Melbourne	UNIMELB	Australia
7	Goeteborgs Universitet	UGOT	Sweden
9	Mrs Nathalie Strazielle	Brain-i	France
10	Applied BioTechnology	ABT	Austria
11	Universitaetsklinikum Wuerzburg	UKW	Germany

Project website

http://www.neurobid.eu