EUROPEAN COMMISSION

Community Research

SEVENTH FRAMEWORK PROGRAMME

TECHNICAL REVIEW REPORT

Grant Agreement number: FP7 202167

Project Acronym: NeuroGLIA

Project title: Molecular and cellular investigation of neuron-astroglia interactions: Understanding brain function and dysfunction

Funding Scheme:

Project starting date: 01/01/2008

Project duration: 4 years

Name of the scientific representative of the project's coordinator and organisation: Christian Steinhäusser

Period covered by the report, from 01/01/2008 to 30/06/2009
Date of review meeting (if applicable): October 12, 2009

Name(s) of expert(s):
- Audinat Etienne

Name of expert drafting the report: Audinat Etienne

Individual report X

Consolidated report ☐

1. OVERALL ASSESSMENT

a. Executive summary

Comments, in particular highlighting the scientific/technical achievements of the project, its contribution to the State of the Art and its impact:

The overall goal of the Neuroglia project is to deliver new knowledge on some of the fundamental mechanisms of interactions between neurons and glial cells, mainly astroglial cells, in the healthy and epileptic brain. The strategy is to use state-of-the-art multidisciplinary functional analyses applied on different animal (rodent) models as well as on human samples obtained after surgery of epileptic patients. The strengths of the project are: first, the association of some of the best european specialists of neuron-glia signalling analyses; second, their access to an amazing number of transgenic animal models, as well as to living human tissue; third, their will to collaborate actively on a number of clearly identified issues, in particular the role of glial cells and inflammation in epilepsy.
Specifically, six major objectives, corresponding to the six scientific workpackages (WPs), are pursued with this project. In the following paragraphs I will briefly summarize the objectives and the achievements after 18 months of each of these WPs.

WP1: The goal is to perform an integrated characterization of astroglia cell subtypes with respect to neuron-glia interactions in different brain areas, during normal brain development and in the epileptic brain.
Results:
As reported in *deliverable1.1*, the potassium channel subunit Kir4.1 has been identified as a key component, together with TREK1 and TREK2 channels, underlying the passive currents of hippocampal astrocytes. Heterogeneity between hippocampal and cortical astrocytes in terms of functional expression of purinergic P2X receptors has been also revealed. Two transgenic mouse lines (Cx43ki-ECFP; NG2ki-EYFP) have been identified and used to study different populations of astroglial cells. The generation of double transgenic mice resulting from these two lines will allow the functional study of identified astrocytes and NG2 cells in same animals. The consortium has also produced evidence for a key role of connexion 43 expressed by radial glial cells of the dendate gyrus in postnatal neurogenesis.
Other progresses within WP1 include the study of the heterogeneity of astrocytes and NG2 cells in the hippocampus and in the thalamus and that of NG2 cells in the cerebellum. GABA-A receptor subunits and of the voltage-dependant calcium channels expressed by NG2 cells have been also identified. Finally, a new mouse model of epilepsy has been developed (unilateral intra-cortical injection of kainate) which mimics key features of human sclerotic temporal lobe epilepsy and which will allow the systematic investigation of astroglial heterogeneity in epilepsy.
There have been no deviations from the original workplan and the results presented in the written report and during the meeting are extremely convincing. Several original papers have been published in which the support provided by the NeuroGLIA project is acknowledged (PNAS, J Neurosci., Glia…). I believe that all the experiments planed in WP1 will be performed on time and that the results produced by this WP will have an important impact in the field and will help the identification of astroglial diversity and therefore the study of glial-interactions.

WP2: This WP aims at identifying the molecular signalling pathways from neuron-to-astroglia and astroglia-to-neuron in situ and in vivo.
Results: Two important sets of data have been already obtained. The first concerns a novel communication pathway between neurons and astrocytes in the hippocampus. Using hippocampal slices, it has been shown that the release of endocannabinoids by neurons activates CB1 receptors expressed by astrocytes and triggers a phopholipase C-dependant calcium mobilization from internal stores. This activation calcium signalling can in turn trigger the release of glutamate from astrocytes which in turn activates neuronal glutamate receptors of the NMDA type. Furthermore, using the same preparation and following previous results showing that activation of astrocytes can lead to a novel form of long term potentiation (LTP) of excitatory synaptic transmission (Perea & Araque, Science 2007), preliminary data presented

in the periodic report indicate that this form of LTP is independent of NMDA receptor activation and involves metabotropic glutamate receptors and production of nitric oxide (NO).
The second set of data is related to the study and the manipulation of astroglia in vivo. First, a two-photon microscope has been constructed and optimized for calcium imaging in vivo. Second, conditional KO mice for invalidating glutamate receptors in vivo have been used to study the role of these receptors in Bergmann glial cells (BG) in the cerebellum. The results show that upon invalidation of these receptors, the thin processes of BG that normally enwrap excitatory synapses regress and these modifications lead to changes in the mice motor behaviour controlled by the cerebellar network.
The results obtained in the frame of this WP have led to the publication of one original article (Neuron, 2008). The preliminary data presented during the review meeting are also very promising and should guarantee the success of this part of the project. There have been no deviations from the original workplan.

WP3: The goal of this WP is to study temporal and spatial dynamics of neuron-glia interactions in the normal and epileptic brain.
Results: A functional abnormality of one the GABA transporters (GAT-1) present in thalamic astrocytes has been identified in different genetic models of absence epilepsy (AE). This loss of function leads to an enhanced tonic inhibition of neurons in specific nuclei of the thalamus. Pharmacological as well as knock-down experiments confirmed the causal link between the loss of function of GAT-1, the increased tonic inhibition and the appearance of AE. These results are of paramount importance for the development of new treatments of AE in humans.
Concerning temporal lobe epilepsy, the consortium has also provided convincing evidence for the involvement of astrocytes in ictal but not in inter-ictal discharges *in vitro*. These results have been obtained by combining electrophysiology and calcium imaging recordings in acute slices as well as in the perfused whole brain preparation and by developing a new and promising model for studying the origin of epileptiform activities in vitro.
Preliminary data on the use of model mice knocked out for connexins and on the involvement of astrocytic CB1 receptors in epilepsy have been also obtained.
The results on GAT-1 in AE have been accepted for publication in Nature Medicine and will have a major impact. There has been only a minor deviation from the original workplan which concerns the change of mouse line to study AE.

WP4: The aim is to identify the role of astroglia in neurovascular coupling in the normal and epileptic brain.
Results: As reported in *deliverable 4.2*, the main finding obtained within the frame of this WP concerns the description of calcium signals in astrocytic endfeet during ictal but not during interictal discharges observed in vitro. Calcium responses in astrocytic endfeet in vitro are accompanied by blood vessel responses which consist in either constriction or dilation depending upon the initial tone of the vessel. The difference in astrocytic endfoot behaviour during ictal and interictal event is unexpected and potentially extremely important for both the outcome of the pathology and the interpretation of fMRI analyses. As discussed during the Amserdam Review meeting, it would be also important, although quite challenging, to confirm these data in vivo using two-photon microscopy.
The mediators of blood vessel responses induced by astrocyte stimulation have been also investigated and, as in the normal brain, the results point toward the involvement of 20-HETE for vasoconstriction during epileptic discharges. The mediator responsible for vasodilation in the epileptic brain remains to be identified. Many pharmacological pathways have been tested with no success. I would suggest the use of transgenic mice in which the production putative mediators has been invalidated.
Tools and animals for the study of neurovascular coupling during AE in vivo and for the study of human potassium channels expressed at the interface between endfeet and vessel vasculature

have been also developed during this first period of the project.
There have been no major deviations from the original workplan except the need of buying a new confocal microscope to replace an old one which had a problem with the scan head. This WP is extremely challenging. Interesting results have been already obtained. However, in vitro preparations might be limited for studying vascular responses and the consortium might favour in vivo approaches for the continuation of this WP.

WP5: This WP is designed to evaluate the role of microglia anf astroglia in the inflammatory response associated with neurodegeneration and hyperexcitability.
Results: Different signalling pathways have been investigated in human and in animal models. As summarized in *deliverable 5.1* the results support a major role of VEGF and its related receptors in astroglia and microglia of human epileptic patients with malformation of cortical development (MCD) and the involvement of plasminogen activators in different forms of human epilepsy. The role of COX-2 has been also investigated but despite its up-regulation after seizures, its inhibition in animal models does not block the development of epilepsy or of seizures. Interestingly though, COX-2 might be involved in the pharmaco-resistance to anti-epileptic drugs due to its action on P-glycoprotein expression.
In addition, a new pathway involving TLR4-HMGB1 interactions has been studied and seems to be proconvulsant in animal models of TLE. Interestingly, TLR4 which has been for long time identified as a specific microglia receptor is now found in astrocytes and neurons of the epileptic brain.
Collaboration between the different members of the consortium to study the effects of inflammation on neuron-glia interactions have also started.
Several articles have been already published (Act. Neuropathol., Epilepsy Res.) but clearly the most interesting parts of this WP will arise from the experiments planned during the second part of the project. There have been no deviations from the original workplan.

WP6: This last scientific WP is dedicated to the analysis of neuron-astroglia signalling in living human brain tissue.
Results: Experiments described in deliverable 6.1 indicate that in the human sclerotic hippocampus (HS), astrocytes vanish whereas cells with typical properties of NG2 (or GluR or complex) cells remain. This process is paralleled by a loss of function of glutamate transporters and of dye coupling in astroglial cells. The hypothesis of a de-differentiation of astrocytes into GluR cells is supported by recent experiments of fate mapping in a new animal model of TLE. In addition, results obtained in human brain slices indicate the occurrence of spontaneous calcium signal in astrocytes and of astrocytic release of glutamate able to activate neuronal NMDA receptors. These experiments represent a "tour de force" and, to my knowledge, are the first favouring the existence of gliotransmission in human brain.
There have been no deviations from the original workplan and the following tasks concerning the electrophysiological characterization of human astroglia heterogeneity and the control of vasculature by human astrocytes should be completed on time.

☐ **Excellent progress (the project has fully achieved its objectives and technical goals for the period and has even exceeded expectations).**

b. Overall recommendations (e.g. on overall modifications, corrective actions at WP level, or re-tuning the objectives to optimise the impact or keep up with the State of the Art, or for other reasons, like best use of resources, re-focusing…).

The objectives of this first period of the project have been fully achieved. There is no reason to change the overall workplan.
I have only one minor concern which is on WP4. As mentioned above, this WP might profit from dropping the pharmacological and in vitro approaches. However, the genetic and in vivo alternative approaches are also risky. This issue may need to be examined in details at the next review report.
Although actual collaborations between the different partners have already started, it is clear that this collaborative efforts will become visible in terms of publications and impact during the forthcoming one or two years. The complementarity of the partners is excellent and their collaboration will continue to generate interesting findings and probably unexpected ones. At this point, probably at the second intermediate reports, it may become necessary to re-tune the objectives to optimise the impact of the whole project.

2. OBJECTIVES and WORKPLAN

a. Have the objectives for the period been achieved? In particular, has the project as a whole been making satisfactory progress in relation to the Description of Work (Annex I to the grant agreement)?

☐ **Yes**

Comments

There have been no deviation from the original workplan and the project achievements reported for this first period compares favourably with the Description of Work presented 2 years ago (Annex 1 of the grant agreement).

b. Has each work package (WP) been making satisfactory progress in relation to the Description of Work (Annex I of the grant agreement)?

☐ **Yes**

Comments

Each workpackage has done satisfactory progress in relation to the initial description of work found in annex I. The only minor deviations concern a change of mouse line and the necessity to buy a new confocal microscope.

c. Have planned milestones and deliverables been achieved for the reporting period?

☐ **Yes**

Comments

No comment

DELIVERABLES LIST STATUS			
No.	**Title**	**Status (Approved/Rejected)**	**Remarks**
D1.1		**Approved**	
D4.2		**Approved**	
D5.1		**Approved**	
D6.1		**Approved**	
D7.1.1		**Approved**	
D7.2.3		**Approved**	

d. Are the objectives for the coming period(s) i) still relevant and ii) still achievable within the time and resources available to the project?

i ☐ **Yes**

ii ☐ **Yes**

Comments

There is no doubt that the objectives for the coming periods are still relevant and can be achieved within the time and resources available to the project.

e. For Networks of Excellence (NoEs) only:

Has the Joint Programme of Activities been realised for the period, with all activities foreseen satisfactorily completed?

☐	☐	☐
Yes	Partially	No

Comments

3. RESOURCES

a. To the best of your estimate, have resources used, i.e. personnel resources and other major cost items, been (i) utilised for achieving the progress, (ii) in a manner consistent with the principle of economy, efficiency and effectiveness. Note that both aspects (i) and (ii) have to be covered in the answer.

i ☐

Yes

ii ☐

Yes

Comments

The most important part of the budget, for all partners, goes to personnel costs. Overall, the budget and resources seem justified in relation with the number of objectives and of man/months to achieve these objectives. There re no obvious indications that the project would have been realised in a manner inconsistent with the principle of economy and efficency.

b. If applicable, please comment on large deviations with respect to the planned resources.

Comments

The only deviation concerns the necessity of buying a new confocal microscope for CNR and is convincingly justified.

4. IMPLEMENTATION OF THE PROJECT

a. Has the project management been performed as required?

☐ **Yes**

Comments

The fact that there are been no deviations from the original workplan is the proof that the scientific management of the project has been well performed. The administrative management performed by Eurice has been also acknowledged as very professional and helpful by all the partners during the reviewing meeting.

b. Has the collaboration between the beneficiaries been effective?

☐ **Yes**

Comments

This one of the specific strength of the project and I think that this will become more and more visible as the project will develop.

c. Do you identify evidence of underperforming beneficiaries, lack of commitment or change of interest of any beneficiaries?

☐ Yes ☐ Partially ☐ **No**

Comments

No comment

5. USE AND DISSEMINATION OF FOREGROUND

a. Is there evidence that the project has/will produce significant scientific, technical, commercial, social, or environmental impacts (where applicable)?

☐ **Yes**

Comments

As reported in point 1, the scientific impact of the project is already important on the basis of the already published results. The preliminary data presented in the periodic report are also very encouraging. Furthermore, some of the results will also have an impact in terms of identification of new therapeutic targets (WP3, 4 and 5) and from this point of view will have social and commercial impacts.

b. Is the plan for the use of foreground, including any update, appropriate? Namely, please comment on the plan for the exploitation and use of foreground for the consortium as a whole, or for individual beneficiary or groups of beneficiaries and its progress to date.

☐ Yes

Comments

Deliverable D7.1.1. shows the lists of publications and of the planned publications in the form of abstracts as it appears in the restricted area of the NeuroGlia homepage. It shows also the list of the meetings already organized within the consortium (3).

c. Have the beneficiaries disseminated project results and information adequately (publications, conferences…)?

☐ **Yes**

Comments

14 peer reviewed articles and 3 articles addressing a broader public have already been published and, for some of them, in the very best journal of the field (Nature Medicine, Neuron, PNAS, J Neurosci). Members of the consortium but also the consortium as a whole have organized and will organize several scientific symposia and conferences.

d. Are potential users and other stakeholders (outside the consortium) suitably involved (if applicable)?

☐ Yes ☐ Partially ☐ No

Comments

Not applicable

e. Is the consortium interacting in a satisfactory manner with other related Framework Programme projects or other R&D national/international programmes, standardisation bodies (if relevant)?

☐ Yes ☐ Partially ☐ No

Comments

Not relevant

6. OTHER ISSUES

a. Have policy-related and/or regulatory issues been properly handled (if applicable)?

☐ Yes ☐ Partially ☐ No

Comments

Not applicable

b. Have ethical issues been appropriately handled (if applicable)?

☐ **Yes**

Comments

There is no specific comments in the first periodic report concerning ethical issues but these points were clearly explained and to the best of my knowledge properly handled in part B of the Annex 1 "Description of work".

c. Have safety issues been properly handled (if applicable)?

☐ Yes

Comments

Same as above.

d. Has progress on Gender Equality Actions been satisfactory (if applicable for this reporting period)?

☐ Yes ☐ Partially ☐ No

Comments

Not applicable

Name (s) of the expert(s): Audinat Etienne

Date: October 27, 2009

Signature(s):