Laboratoire de neurophysiologie et nouvelles microsopies

Rapport Hcéres

agence d'évaluation de la recherche
et de l'enseignement supérieur

Department for the evaluation of research units

AERES report on unit:

Laboratory of Neurophysiology and New Microscopies

Under the supervision of the following institutions and research bodies:

Université Paris Descartes

Institut National de la Santé Et de la Recherche Médicale

January 2013

agence d'évaluation de la recherche
et de l'enseignement supérieur

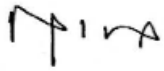
Research Units Department

President of AERES

Didier Houssin

Research Units Department

Department Head



Pierre Glaudes

The president of AERES "signs[...], the evaluation reports, [...]countersigned for each evaluation department by the relevant head of department (Article 9, paragraph 3, of french decree n°2006-1334 dated 3 november 2006, revised).

Grading

Once the visits for the 2012-2013 evaluation campaign had been completed, the chairpersons of the expert committees, who met per disciplinary group, proceeded to attribute a score to the research units in their group (and, when necessary, for these units' in-house teams).
This score (A+, A, B, C) concerned each of the six criteria defined by the AERES.
NN (not-scored) attached to a criteria indicate that this one was not applicable to the particular case of this research unit or this team.

Criterion 1 - C1 : Scientific outputs and quality ;
Criterion 2 - C2 : Academic reputation and appeal ;
Criterion 3 - C3 : Interactions with the social, economic and cultural environment ;
Criterion 4 - C4 : Organisation and life of the institution (or of the team) ;
Criterion 5 - C5 : Involvement in training through research ;
Criterion 6 - C6 : Strategy and five-year plan.

With respect to this score, the research unit concerned by this report and its in-house teams received the following grades:

- Grading table of the unit: Laboratory of Neurophysiology and New Microscopies

C1	C2	C3	C4	C5	C6
A+	A+	B	A	A	A+

- Grading table of the team: From sensory processing to functional hyperemia

C1	C2	C3	C4	C5	C6
A+	A+	A	NN	NN	A+

- Grading table of the team: Neuron-glia interactions

C1	C2	C3	C4	C5	C6
A	A	B	NN	NN	A+

- Grading table of the team: Physiology of NG2 cells

C1	C2	C3	C4	C5	C6
A	A	NN	NN	NN	A+

Evaluation report

Unit name:	Laboratory of Neurophysiology and New Microscopies
Unit acronym:	
Label requested:	INSERM Unit
Present no.:	INSERM U603 CNRS UMR 8154
Name of Director (2012-2013):	Mr Serge CHARPAK
Name of Project Leader (2014-2018):	Mr Serge CHARPAK

Expert committee members

Chair:	Mr Daniel CHOQUET (Institut de Neurosciences, Bordeaux)
Experts:	Ms Carinne BOSSENMEYER-POURIE, Nancy, (Representative of CNU)
	Ms Isabelle LOUBINOUX Toulouse, (Representative of INSERM CSS)
	Mr Christian STEINHÄUSER, Institute of Cellular Neurosciences, Bonn, Germany
	Mr Bruno WEBER, Institute of Pharmacology & Toxicology, University of Zurich, Switzerland

Scientific delegate representing the AERES:

Jacques HAIECH

Representative(s) of the unit's supervising institutions and bodies:

Ms Catherine LABBÉ-JULLIÉ and Stefano MARULLO (Paris 5)

Ms Marie-Josèphe LEROY-ZAMIA (INSERM)

1 • Introduction

History and geographical location of the unit

The laboratory of Neurophysiology and New Microscopies was initially created in 1995 at ESPCI and comprised physicists and neurophysiologists. Since they moved to the University Paris Descartes, at the Faculté des St-Pères de Médecine, the laboratory has been supported as a Research Unit by the INSERM (as U603), the CNRS (UMR 8154) and the University.At the moment, the laboratory is structured in 5 independent teams, 4 teams of neurophysiologists and 1 of physicists.

The last team, headed by Ms Valentina EMILIANI, joined the unit at the end of 2005 and was strongly supported by the laboratory which attributed to the team the only research position that they obtained from the University. During the last four years, the team has succeeded to impose itself nationally and internationally as one of the best in the use of wavefront-engineering microscopy applied to Neuroscience.The University has thus decided to launch a Department of Physics under the direction of Ms Valentina EMILIANI, by renovating large laboratory surfaces and creating several positions of assistant professorships and full professorships.This move of the University can be considered as a success for the current Unit. Because of this overall evolution, the proposers decided to split the INSERM/CNRS Unit in two laboratories, for the next five years: Mr Serge CHARPAK will continue to head the Laboratory of Neurophysiology and New Microscopies but with the sole support of INSERM, while Ms Valentina EMILIANI will head the new Laboratory of Wavefront-Engineering Microscopy, with the sole support of the CNRS.

Finally, the two teams which have been temporarily associated to the Laboratory for administrative reasons, are not discussed in our evaluation, as they have never been part, scientifically, of the current Laboratory.

Management team

The unit is directed by Mr Serge CHARPAK.The governing principle is independency for the different teams.The laboratory is managed by regular meetings of the PIs. During the visit, it was noted that the lab council is not meeting frequently enough, and as a consequence, technicians are not sufficiently involved in decision making procedures.

The future Laboratory of Neurophysiology and New Microscopies will comprise three small teams: each team will be headed by a permanent researcher, Mr Serge CHARPAK, Mr Etienne AUDINAt and Ms María Cecilia ANGULO, and will have its research performed by PhD students and postdoctorants, in addition to research engineers.

In the new setting, and in particular due to the reduction in size of the unit, an increased involvement of all personnel to decision making will be implemented.

PhD students and post-docs expressed a lack of involvement in teaching duties and the wish to participate to a higher number of international conferences. A compilation of all conference participations by postdocs and PhD students prepared by Mr Serge CHARPAK after the committee meeting however does not confirm an inadequate number of conference attendence.These points are planned as well to be improved in the new setting of the unit.

AERES nomenclature

SVE1_LS5

ST2/5

Unit workforce:

Unit workforce	Number as at 30/06/2012	Number as at 01/01/2014	2014-2018 Number of project producers
N1: Permanent professors and similar positions	3		
N2: Permanent researchers from Institutions and similar positions	9	3	3
N3: Other permanent staff (without research duties)	6	5	
N4: Other professors (Emeritus Professor, on-contract Professor, etc.)			
N5: Other researchers from Institutions (Emeritus Research Director, Postdoctoral students, visitors, etc.)	5	5	5
N6: Other contractual staff (without research duties)	14	10	
TOTAL N1 to N6	37	23	8

Percentage of producers	***100 %***

Unit workforce	Number as at 30/06/2012	Number as at 01/01/2014
Doctoral students	6	
Theses defended	5	
Postdoctoral students having spent at least 12 months in the unit*	5	
Number of Research Supervisor Qualifications (HDR) taken	3	
Qualified research supervisors (with an HDR) or similar positions	3	3

2 • Assessment of the unit

Strengths and opportunities

The main strength of this unit has always been, and will continue to be, interdisciplinarity through the development of new innovative imaging methods applied to answer key questions in neuroscience.

Several common competences and goals are shared by the 3 teams. All of them use, or develop, imaging tools to answer biological questions. All teams have investigated some aspects of neuronal and glial neurophysiology. The three teams share a strong background in electrophysiology and imaging.

In the new unit, several new collaborations are planned. Mr Etienne AUDINAT and Mr Serge CHARPAK will image both calcium signaling and motility of microglial cells in the developing barrel cortex, while Ms Maria Cecilia ANGULO and Mr Serge CHARPAK will image calcium signaling of NG2 cells. This joint focus will be extremely beneficial for this small unit which can be extremely reactive.

Altogether, the unit has had a very good publication record and a very good international visibility and attractivity as exemplified by the success to funding applications and attraction of foreign post-docs.

Weaknesses and threats

A threat is the decrease in size going from 5 teams to 3 teams as support needs to be granted for administration, animal facility, instrumentation, every day lab running, etc.Given the relative isolation of the unit, allocation of resources by the funding bodies may be difficult to obtain.

International collaborations could be increased.

The animal house facilities are still suboptimal although they have been improved.

Above all, technical support for basic tasks is lacking.

Recommendations

In accordance with the University, the unit plans to recruit a fourth young independent team within the next 1-3 years.This is important in order to increase the critical mass of the unit.Using for example the opportunity of the mechanism of chairs of excellence, assistant professor or professor position would allow attracting candidates of highest level.

The project of the unit, which is based on small independent teams, would well fit in a future Department of Neuroscience which is locally planned for the next few years.This would in particular allow better sharing of common resources for laboratory management and core facilities such as the animal facility that is still lacking personnel.

In terms of current organization of the unit, there is room for improvement of the participation of the technical personnel to the operational decisions through more regular meetings of the lab council.

PhDs and post-docs could be offered more opportunities to participate to teaching and mentoring to improve their professional experience.

The post-docs and PhDs should be encouraged and supported to present and discuss the data at international conferences to help them build their scientific network.

3 • Detailed assessments

Assessment of scientific quality and outputs

The unit aims at understanding basic mechanisms of brain function using a variety of approaches and a particular strength on imaging and in vivo physiologiocal approaches.The research has an emphasis on neuron function with respect to its environment, glia and blood vessels.It also analyses brain function during development or how it is altered in several pathological conditions such as hypoxia, epilepsy and demyelination. However, the unit is not directly involved in clinical research, but favours the translation of its results to the medical community, as exemplified by the collaboration that team 2 has already launched by studying human epileptic tissue and by the collaboration that Mr Serge CHARPAK is beginning with the fMRI imaging community.

The unit is organized in 3 independent teams with pronounced interactions:

1) Mr Serge CHARPAK: Sensory processing to functional hyperemia
2) Mr Etienne AUDINAT: Neuron-glia interactions
3) Ms Maria-Cecilia ANGULO: Physiology of NG2 cells

All three teams have a very good international recognition, the team of Ms Maria-Cecilia ANGULO beeing the youngest coming to independence only 2 years ago.The team of Mr Serge CHARPAK is particularly well recognized at the international level due to major ouputs since several years in the field of in vivo imaging and more recently its application to imaging local neuronal activity by monitoring pO2 transients in capillaries.The team of Mr Etienne AUDINAT has very good recognition in the field of neuro-glia interaction with important contribution on the role of microglial cells during the normal postnatal development of the somatosensory cortex or during epilepsy.The team of Ms Maria-Cecilia ANGULO is setting itself as an expert of NG2 cells and is a very promissing young team.

Altogether the three teams have made excellent production during the last period, with numerous papers with IF > 5. (J. Neurosci. (8), Nat. Methods (1), Nature (1), Nat. Medicine (2), PNAS (1), Progress in Neurobiol. (1), Glia (1)).

All group leaders have regular invitations to international meetings, including Gordon conferences.

Altogether, this is a small, albeit very well organized and productive unit.

Assessment of the unit's academic reputation and appeal

The academic reputation of the unit is very good as exemplified by the high number of post-docs as well as the very good level of funding

Assessment of the unit's interaction with the social, economic and cultural environment

The unit has some involvement in technological transfert. It bears 1 patent.

It is part of the ENC “Ecole des Neurosciences de Paris”.

One team leader is General Secretary of the French Society for Neuroscience.

Assessment of the unit's organisation and life

The Mr Serge CHARPAK’s unit organizes weekly seminars, namely (i) weekly progress reports of PhD students; ii) weekly seminar in optics.In addition, members of the unit participate in 2 two-weekly seminars running at faculty of St Peres.There is a policy that all researchers and students attend at least one international conference per year. Technicians receive continuous training.There is a rule that one permanent researcher can train up to two PhD students. The unit administrative organisation has been based on weekly meeting between team leaders, each team leader also organising its own group meeting every week.

The unit urgently needs a technician (glassware cleaning, basic ordering, parcel collection, ...) and a technical engineer responsible of immunochemistry, genotyping, virus amplification,electroporation.There is always a need for improvement of animal facilities, already noted in the previous evaluation.

Assessment of the unit's involvement in training through research

The laboratory is involved in teaching, albeit at a rather low level.Researchers teach at Ecole Normale Supérieure (ENS), Universities Paris Descartes and Pierre et Marie Curie for Master 1 and 2 students.The three principal investigators are full time researchers.

The PhDs are affiliated to three different doctoral school, namely ED 158 Cerveau, Cognition, Comportement (3c), ED 157 Génétique, Cellulaire, Immunologie, Infectiologie et Développement (Gc2id) and ED 474 Frontières Du Vivant.

Assessment of the five-year plan and strategy

The 5 year scientific strategy is excellent.The overall plan of individal teams is reinforced by 2 major inter-team collaborations in the field of calcium imaging of glia that could allow to strengthen the position of this unit at the forefront of integrated brain physiology, in particular of the role of glial cells in their environement.

In terms of organization, the perspectives would be the recruitment of a new team and the building of a neuroscience department that would very much help the overall organization of the unit.One important question is the type of support that would be available for a new team.

4 • Team-by-team analysis

Team 1 : From sensory processing to functional hyperemia

Name of team leader: Mr Serge CHARPAK

Workforce

Team workforce	Number as at 30/06/2012	Number as at 01/01/2014	2014-2018 Number of project producers
N1: Permanent professors and similar positions			
N2: Permanent EPST or EPIC researchers and similar positions	2	1	1
N3: Other permanent staff (without research duties)	2	1	
N4: Other professors (PREM, ECC, etc.)			
N5: Other EPST or EPIC researchers (DREM, Postdoctoral students, visitors, etc.)	2		
N6: Other contractual staff (without research duties)	1		
TOTAL N1 to N6	7	2	1

Team workforce	Number as at 30/06/2012	Number as at 01/01/2014
Doctoral students	2	
Theses defended	3	
Postdoctoral students having spent at least 12 months in the unit	2	
Number of Research Supervisor Qualifications (HDR) taken	1	
Qualified research supervisors (with an HDR) or similar positions	1	1

• Detailed assessments

Assessment of scientific quality and outputs

The team investigates synaptic transmission, oxygen consumption and functional hyperemia in the olfactory bulb. In addition, the team developed optical imaging technology based on two-photon excitation. The team undoubtedly belongs to the top research laboratories worldwide in the field of neurovascular coupling and also of information processing in the olfactory bulb. The publication record of the group is excellent, with several papers in the highest-ranking papers in neuroscience, including Nature (1), Nature Medicine (2), Nature Methods (1), Neuron (1), Journal of Neuroscience (6), Proceedings of the National Academy of Science of the USA (1). In the field of neurovascular coupling, Mr Serge CHARPAK was among the pioneers applying the newly developed two-photon excited phosphorescence lifetime probes to measure blood and tissue oxygen partial pressure (pO2).In two papers published in Nature Medicine, the group could show that pO2 and blood flow increased upon sensory stimulation in capillaries, whereas pO2 in the neuropil first decreased (”initial dip”) and thereafter increased.Interestingly, they observed erythrocyte-associated transients (EATs) at the capillary level associated with each individual erythrocyte. pO2 between erythrocytes was shown to correspond well to the parenchymal pO2.The team leader has been invited numerous times to international conferences and has been invited to comment on recent research highlights (Nature Methods).

Assessment of the team’s academic reputation and appeal

Mr Serge CHARPAK directed the Laboratory of Neurophysiology and New Microscopies during the assessment period. He acts as a member of the UFR Biomedicale Scientific Council and is a member of the Paris School of Neuroscience Scientific Council. In 2008 he was chair of the Jacques MONOD Conference on “Investigating brain function using light”. He has received the Medical Research Foundation Team Award in 2010 and the PhD thesis of one of the team members received the AXA French Science Academy Award in 2011.

The team collaborates and publishes with some of the top neuroscientists. It is continuously supported by third-party funds from national and international funding agencies. Above all, it is part of a transatlantic network of excellence generously funded by the Fondation Leducq (760 kEUR).

Assessment of the team's interaction with the social, economic and cultural environment

The team benefits from an international collaboration with industrial firms (Hilo Imaging) for the development of a new imaging technique wich will allow recording of single cell activity, oxygen consumption and red blood cell flow, simultaneously with acquisition of BOLD signals.

the team developed 1 International patent (WO / 2011 / 023593)

Assessment of the team's involvement in training through research

The laboratory trains doctoral fellows (3) and provides cutting edge setups for their research. 3 PhDs graduated during the assessment period. Mr Serge CHARPAK is furthermore responsible for the teaching session “Electrophysiology and cellular imaging” from the Master 2 of Neurobiology at the University Paris Descartes.

Assessment of the five-year plan and strategy

The team proposes to continue to use the model of the rodent olfactory bulb to investigate aspects of neurovascular coupling. They plan to use a multimodal approach including electrophysiological recordings, two-photon imaging and new optical developments. The proposed research is organized in three interdependent subparts, each tackling specific scientific questions:

Theme 1: Cellular decomposition of neurovascular signaling

1) What is the role of external tufted cells in neurovascular coupling in the olfactory bulb?

The underlying hypothesis of this work package is that external tufted cells play an important role in neurovascular coupling in the olfactory bulb. The team proposes to use a transgenic mouse to selectively express GCaMP3 and halorhodopsin in ET cells (in collaboration with ICM and Yale University).The use of optogenetic silencing of ET cells with halorhodopsin will elucidate the role of this cell type in neurovascular signaling.

2) Neurovascular coupling depends on neuronal and astrocytic signaling pathways. The role of each of the two cell types is yet unresolved and will be further investigated.

Preliminary experiments of the group show that the activity of astrocytes and neurons depends differently on the level of the stimulation intensity. The aim is to use a chronic window preparation to image cells and capillary flow in repeated sessions. The setup will be adapted to allow for drug superfusion. The subproject will be carried out with transgenic mice expressing GCaMP3 in astrocytes (R26-lsl-GCaMP3 provided by external collaborator).One important aspect of this mouse model is the potential discrimination between somatic and process/endfeet related calcium signals in astrocytes.

Theme 2: Oxygen dynamics in the olfactory bulb

In the past, the group has provided very important new insights into local oxygen dynamics. One logical further step is to substantiate the findings in the awake behaving mouse. Although not very specific in their research plan, the team will most probably use head-fixation to acquire phosphorescence lifetime measurements of pO2 in tissue and blood under odor stimulation and different brain states (sleep states, awake state).

Theme 3: Novel optical technology for imaging neuronal activity

1) Scan-less two-photon imaging

A weakness of two-photon laser scanning microscopy is the temporal resolution, which essentially depends on the scanning speed. The group is currently working on a scan-less “encoded multi-site two-photon microscopy (eMS2PM).The group has collected preliminary data with this promising approach, which depends on a multi-beam excitation in combination with a specific binary amplitude modulation sequence that allows decoding site-specifically with a single photo-detector.

2) Simultaneous optical imaging and fMRI

Mr Serge CHARPAK and colleagues propose to simultaneously perform optical imaging and fMRI to further our understanding of the complex BOLD functional Resonance Magnetic Imaging (fMRI) signal.Optical imaging will be performed using HiLo. HiLo imaging is a scan-less technique, which can be adapted for MR-compatibility.I t will be used to follow calcium dynamics in astrocytes and neurons as well as pO2 in single vessels and tissue. Brain activity in the olfactory bulb will be modulated by odor stimulation.

Conclusion

- Strengths and opportunities:

The team is a world leading research group that continues with the proposal of a very ambitious yet completely realistic research plan for the next five years. With the upcoming research, the team will further strengthen its role in research both in olfactory processing and neurovascular coupling. This is also particularly evident, as the group continues to increase its methodological bandwidth (multimodal imaging across spatial scales, novel genetically engineered approaches, etc.).

Financing supports is insured for the next 2,5 years.

- Weaknesses and threats:

The research plan is well balanced and includes subprojects with a varying level of risk/yield ratios. There are no obvious threats attached to the proposed research given adequate support in infrastructure and budget.

The last project "imaging in the magnet" is probably risky but obviously extremely important if successful.

- Recommendations:

From an infrastructure point of view, the team relies on a professional animal facility and a workshop that can provide mechanical designs and prototype production.

Team 2 : Neuron-glia interactions

Name of team leader: Mr. Etienne AUDINAT

Workforce

Team workforce	Number as at 30/06/2012	Number as at 01/01/2014	2014-2018 Number of project producers
N1: Permanent professors and similar positions			
N2: Permanent EPST or EPIC researchers and similar positions	1	1	1
N3: Other permanent staff (without research duties)	1	1	
N4: Other professors (PREM, ECC, etc.)			
N5: Other EPST or EPIC researchers (DREM, Postdoctoral students, visitors, etc.)	1	1	1
N6: Other contractual staff (without research duties)			
TOTAL N1 to N6	3	3	2

Team workforce	Number as at 30/06/2012	Number as at 01/01/2014
Doctoral students	2	
Theses defended		
Postdoctoral students having spent at least 12 months in the unit	1	
Number of Research Supervisor Qualifications (HDR) taken	1	
Qualified research supervisors (with an HDR) or similar positions	1	1

• Detailed assessments

Assessment of scientific quality and outputs

After a former member became in 2010 the independent project leader of team 3, the team headed by Mr Etienne AUDINAT has been focussing on the role of microglia in postnatal development and epilepsy, and on the mechanisms astrocytes used for transmitter release. No doubt, these are very timely and important topics. Within the last 5 years, the team leader has become an internationally well respected scientist in the microglia field, owing to his excellent publications in high-impact journals such as J Neuroscience (3x within the last 5 years), Glia(1) and J Physiol(1). Overall, within the reporting period the team has published 7 original papers, 2 reviews and a commentary. Among the key findings were the detection in a mouse TLE model of a rapid SE-induced activation of microglia and enhanced purinergic signalling, and a detailed characterization of the interplay between microglia and neurons during early postnatal development of the barrel cortex. They could also show that astrocytes in addition to glutamate, release GABA, and quantified with high-resolution microscopy the abundance of GABA expressed by astrocytes. The team leader received many invitations to speak at national and international conferences, among others at a Gordon Research Conference on 'Glial biology' in 2009.

Assessment of the team's academic reputation and appeal

The team leader served as a scientific reviewer in the FP7 program of the EU and at various national scientific committees, including the Governing Council of the French Society for Neuroscience, of which he is the current General Secretary.He has organized national (2008, FNS) and international meetings (2009, sattelite at the European Glia Meeting).

Assessment of the team's interaction with the social, economic and cultural environment

The team collaborates with a number of key leaders in the neurosciences at the national and international (Japan, Spain) levels. It is continuously supported by third-party funds from national funding organizations (e.g. ANR, FFRE).

Assessment of the team's involvement in training through research

The team leader teaches bachelor and master students (6-12 h per year) at 3 Paris Universities (Paris 5, Paris 6 et Paris 7).He has been a member of 15 thesis evaluation committees during the reporting period. Since 2007 4 PhD fellows, 9 master students and 7 lab technicians have been supervised.

Assessment of the five-year plan and strategy

The team will focus on the role of microglia in epilepsy (theme 1) and in the postnatal development of cortical neuronal circuits (theme 2).The first theme seeks to elucidate the mechanisms through which SE-activated microglia and inflammation affect neuronal properties and survival. In this context, it is important to mention that in many types of epilepsy the initiating/causal events are still unknown (cryptogenic), although often juvenile febrile seizures are suspected to represent a predisposing event. Thus, inflammation and microglial activation in the context of epileptogenesis are highly topical fields and the proposed research holds great potential for the development of translational approaches. In goal (a), the role of purinergic receptors and inflammation post SE is investigated. Therefore, transcriptome analysis after SE in control and P2X4R and P2X7R Knockout mice are planned (aim 1) and with the help of the same mouse strains, the role of these receptors in neuronal death post SE will be investigated (aim 2). In the hippocampus, P2X receptor expression is confined to MGCs (Jabs et al., 2007). Aim 3 focuses on inflammation-related changes in hippocampal astrocyte-neuron signalling occurring in the latent period post SE, i.e. before onset of spontaneous generalized seizure activity. Potential alterations in the release of GABA and glutamate from astrocytes and its effect on neuronal synchronization will be monitored. These experiments have a high potential to identify mechanism(s) that are causative for epileptogenesis. Goal (b) is devoted to a better understanding of the impact of SE-induced infiltration of leukocytes through Brain Blood Barrier (BBB) leakage. In aim 1, flow cytometry and histochemistry will be used to characterize the time course of monocyte and lymphocyte infiltration post SE in the systemic kainate model and in human epilepsy surgical specimen. Aims 2 and 3 address i) the impact of leukocyte

infiltration on inflammation, ii) neuronal survival and excitability after epileptic seizure. A battery of complementary molecular and functional analyses will be applied to control mice and those lacking T cells or monocytes.

No doubt that the planned approach is suited to shed new light on the impact of blood cell infiltration on epileptogenesis post SE. Theme 2 addresses a developmental aspect, i.e. the question by which pathways MGCs influence functional maturation of cortical synapses.Building on their own published work, the significance of CX3CR1 for the timing of Long Term potentiation (LTP) induction will be further investigated at thalamo-cortical and cortico-cortical synapses, employing transient depletion of MGCs (with Mac1-saporin) and mice deficient for CX3CR1 (aim 1).In aim 2, the team will elucidate mechanisms through which MGCs affect the regulation of neuronal NMDA receptor expression.A focus will be on the presumed mediators IL-1beta, TNFalpha and IL-6. Finally, the team aims at a better understanding of the link between Ca2+ signaling and motility patterns in MGCs during postnatal development of the barrel cortex. In collaboration with the two other teams, mice specifically expressing GCaMP3 in MGCs and 2P imaging will be employed in vitro and in vivo to investigate spontaneous and activity-induced Ca2+ responses, correlate the responses with MGC movement, and investigate the role of glutamate and purine receptors in the MGC responses. This is a challenging, consistent project that will provide important new insight into the role of MGCs in functional maturation of cortical neural circuits.

Conclusion

- Strengths and opportunities:

The team proposes a challenging and consistent work program to investigate the impact of MGCs in epilepsy and normal cortical maturation.Cutting edge methods will be employed in situ and in vivo, including qRT-PCR, transgenics and high resolution imaging.The team has already a good standing in the field and published several high quality papers.The projects also utilize the specific expertise of the other two teams of the Unit.Theme 1 holds a high potential for continuative translational approaches.There are no doubts that this team will continue its successful research through the next five years.

- Weaknesses and threats:

No significant risks can be identified.Alternative strategies have been outlined in case some of the planned experiments will fail.

- Recommendations:

It is strongly recommended to increase the technical assisatnce of the Unit, which has been apparently lacking for more than two years.

Team 3 : Physiology of NG2 cells

Name of team leader: Ms Maria Cecilia ANGULO

Workforce

Team workforce	Number as at 30/06/2012	Number as at 01/01/2014	2014-2018 Number of project producers
N1: Permanent professors and similar positions			
N2: Permanent EPST or EPIC researchers and similar positions	1	1	1
N3: Other permanent staff (without research duties)			
N4: Other professors (PREM, ECC, etc.)			
N5: Other EPST or EPIC researchers (DREM, Postdoctoral students, visitors, etc.)	2	2	2
N6: Other contractual staff (without research duties)			
TOTAL N1 to N6	3	3	3

Team workforce	Number as at 30/06/2012	Number as at 01/01/2014
Doctoral students	2	
Theses defended	2	
Postdoctoral students having spent at least 12 months in the unit	0	
Number of Research Supervisor Qualifications (HDR) taken	1	
Qualified research supervisors (with an HDR) or similar positions	1	1

• Detailed assessments

Assessment of scientific quality and outputs

This team, which became independent in 2010, is active in an emerging and rapidly evolving field, the physiology of NG2 cells.These cells are unique because they represent the only non-neuronal cell type in the CNS that receives synaptic input from neurons.In white matter, most NG2 cells become oligodendrocytes and can be considered OPCs, but in grey matter a majority of these cells keep their NG2 phenotype throughout life and do not differentiate into another cell type.The function of these cells is still unclear.The team is currently comprised of 2 postdocs and 2 PhD students. Between 2010-2012, the team published 5 articles, in 4 of which Ms Angulo served as a senior author. Among them, three are original articles, and another two invited reviews.The team published its work in leading journals of the field (J Neurosci IF:7.7 cited 8 times, PLoS One IF: 4.4 cited 10 times, Glia IF:4.8 cited 11 times, The Neuroscientist IF: 4.5 cited 4 times, J Anat IF:2.3 cited 3 times and a Progress in Neurobiol IF:8.8 in 2008, first author cited 22 times, a J Physiol-London IF:4.7 cited 54 times).Considering the short time period, this scientific output is remarkable.

Assessment of the team's academic reputation and appeal

Although being active in the NG2 field for a few years only, Ms Angulo is already well visible internationally. She has published high impact data on NG2 physiology (see above).Notably, she has organized a symposium on 'Functional roles of channels, receptors and synapses of NG2 cells' at the 10th European glia meeting (Prague 2011). This symposium not only comprised key players in the field as speakers, but attracted also a great scientific audience (>200 scientists).

The team leader developed national collaborations in Paris which are fruitful (co-signed papers with collaborator from (Institut du Cerveau et de la Moëlle (ICM)).International collaborations are established not only on sharing mouse strains but mainly on common scientific projects.

The level of post-doctorants recruited in the team is good given the well-known journals in which the team publishes.The team is currently supported by grants from national funding organizations.It is is coordinating an FRC project and acquired a postdoc salary from a regional funding body.In addition, it is supported as a co-PI from ANR and ARSEP grant.

Students got prizes from the French Society for Neuroscience (2011) and the best oral communication in the 23rd ion channel meeting.

Assessment of the team's interaction with the social, economic and cultural environment

This criteria is not applicable for this team.

Assessment of the team's involvement in training through research

Since 2010 2 PhD students and 3 master2 students have been supervised.Two lab tech students have been trained in the team. One of the PhD students received in 2011 a thesis award from the French Society for Neuroscience. Ms Angulo is teaching master students at Ecole Normale Superieure and in 2 Parisian Universities (Université Pierre et Marie Curie and Université Paris Descartes).

Assessment of the five-year plan and strategy

The team will focus on two themes of high current interest: i) analysis of interneuron-NG2 cell interactions in the somatosensory cortex and ii) investigation of the glutamatergic input onto NG2 cells in the corpus callosum. In theme i), the first goal (a) is to investigate GABAergic input on NG2 cells with holographic photolysis of MNI glutamate, which is certainly a promising approach to search for the (relatively few) synapses connecting both cell types. This paragraph is method-centered and lacks a clear scientific question/hypothesis, although developmental studies were mentioned in its heading. Viral approaches to knock down GABA receptor subunits are shortly mentioned. This might prove difficult, given the obvious problems with virus-based NG2 cell infections in vivo. In goal (b), it is

intended to investigate interneuron-NG2 cell signalling after perinatal hypoxia (PH), to better understand the potential role of the neuronal input on glial proliferation and differentiation.

It is argued that the reduction of interneuron density will affect NG2 cell activity. This approach appears not completely convincing because PH may also affect NG2 cells through mechanisms other than altered GABAergic input. What is exactly meant by the term 'reacted NG2 cells' remains unclear. It is not clear either who will perform the planned EM analyses. Finally, (goal c) two-photon based Ca2+ imaging experiments are planned in vivo, in collaboration with the two others teams.

The approach is well structured and addresses a very important open question. Since by now clear evidence for the existence of synaptic input-induced Ca2+ signals in NG2 cells is lacking, this part of the project is risky. Before planning to investigate presumed changes in activity-induced Ca2+ signalling in PH, the signals have to be identified under control conditions (which is still outstanding). Since the team did not start the experiments on PH yet and because the feasibility is not guaranteed, this part of the project is not put forward for the moment.

Theme ii) addresses two questions: a) does glutamatergic synaptic onto NG2 cells in white matter impact proliferation and differentiation, and b) does it affect remyelination. In goal a) lentiviral infection is planned to knock down GRIP1, a protein that interacts with the GluR2 subunit which is most abundant in NG2 cells. The proposed loss of function effect will be investigated at different developmental stages at axonal-NG2 cell synapses, after injection of lentivirus-mediated shRNA. In addition, a gain of function approach is planned, using optogenetic lentiviral vectors. This is a very interesting project. However, no evidence is provided demonstrating the feasibility of lentiviral infection in vivo (cf. above). The collaborating group has published successful lentiviral transfection of OPCs in culture, but apparently not in vivo. In goal b), the group will investigate which alterations NG2 cells undergo in lysolecithin-induced demyelination. Finally, the impact of photostimulated neuronal fibers projecting into the corpus callosum onto NG2 cells in demyelinated areas will be investigated. It is hypothesized that axon photostimulation affects proliferation and differentiation of NG2 cells, and possibly myelination.

Conclusion

- Strengths and opportunities:

The team provides a challenging and timely work program to investigate the impact of synaptic innervation onto NG2 cells in two selected brain regions, the somatosensory cortex and the corpus callosum. Cutting edge methods will be employed in situ and in vivo, including optogenetics, transgenics and high resolution imaging. The strategy to learn more about the physiological impact of these cells through loss of function, gain of function and pathological modifications is promising.

- Weaknesses and threats:

Parts of the work program appear somewhat risky. For example, to see modified activity-dependent Ca2+ responses in NG2 cells after PH, a first step would be to demonstrate such signals under control condition which, however, is still outstanding. The planned lentiviral experiments critically depend on the ability to infect NG2 cells in vivo. This proof of principle is still outstanding.

- Recommendations:

More intense international collaborations could be initiated.

5 • Conduct of the visit

Visit date:

Start: January 7th, 2013 at 8h30

End: January 7th, 2013 at 18h

Visit site(s):

Institution: Laboratoire de Neurobiologie et Nouvelles microscopies

Address: 49, rue Jacob Paris 75006

Specific premises visited:

See program of the visit

Conduct or programme of visit:

8:30 to 8:45	Welcome brunch
Door-closed meeting	Presentation of the AERES to the Committee members
9:15 to 9:30	Presentation of the Committee and of the AERES to the Unit
9:30 to 10:00	Mr Serge CHARPAK : Past activity and projects of the Unit
10:00 to 10:30	Team #1 (Mr Serge CHARPAK)
10:30 to 11:00	Team #2 (Mr Etienne AUDINAT)
11:00 to 11:30	Team #3 (Ms Maria Cecilia ANGULO)
11:30 to 11:45	Coffee break
11:45 to 12:15	Two simultaneous meetings (The committee will split in two) : Meeting with PhD students and postdoctoral fellows Meeting with engineers, technicians and administrative assistants
12:15 to 13:15	Lunch break
	Afternoon session: 3rd Floor, rue des Saints-Pères
13:15 to 14:45	Lab and team visits, poster exhibits
Door-closed meeting:	Committee members, representatives of University and Research Organizations
15:15 to 16:00	Door-closed meeting: Committee members ± Lab director
Final Door-closed meeting	Committee members

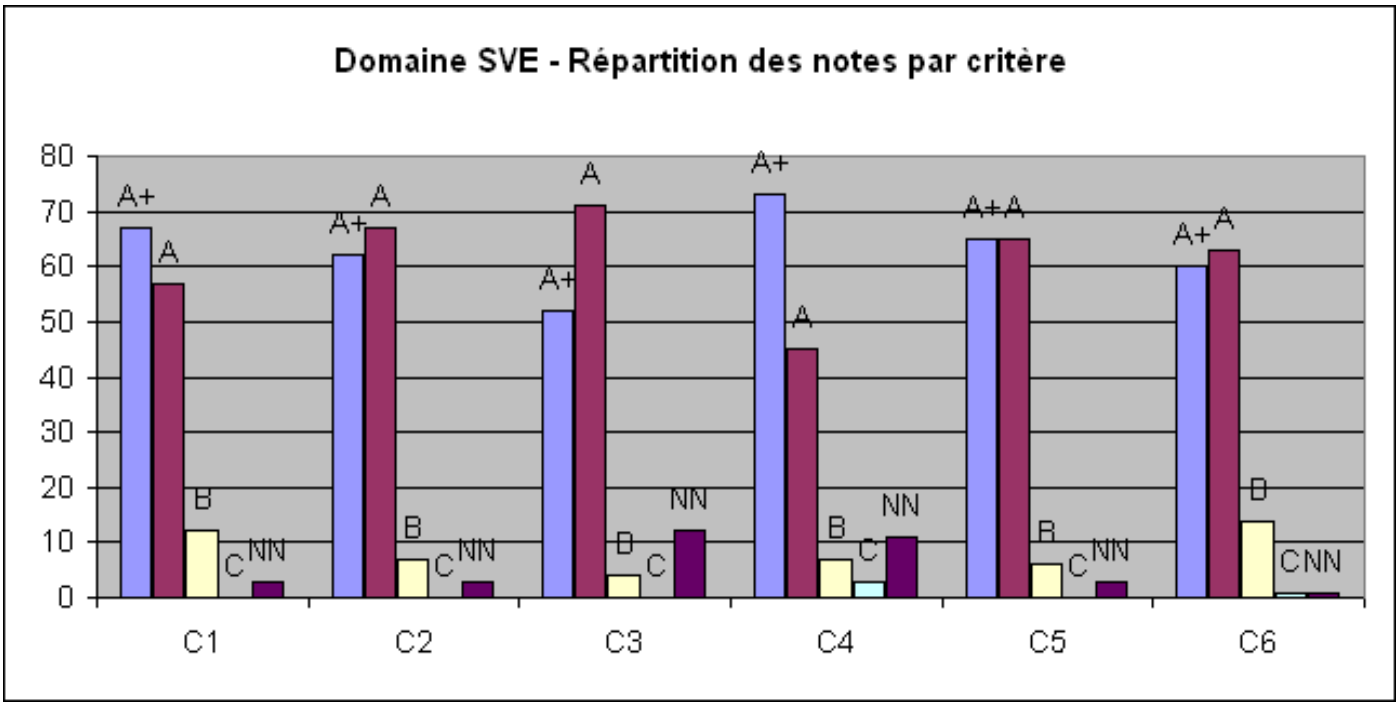
6 • Statistics by field: SVE on 10/06/2013

Notes

Critères	C1 Qualité scientifique et production	C2 Rayonnement et attractivité académiques	C3 Relations avec l'environnement social, économique et culturel	C4 Organisation et vie de l'entité	C5 Implication dans la formation par la recherche	C6 Stratégie et projet à cinq ans
A+	67	62	52	73	65	60
A	57	67	71	45	65	63
B	12	7	4	7	6	14
C	0	0	0	3	0	1
Non Noté	3	3	12	11	3	1

Pourcentages

Critères	C1 Qualité scientifique et production	C2 Rayonnement et attractivité académiques	C3 Relations avec l'environnement social, économique et culturel	C4 Organisation et vie de l'entité	C5 Implication dans la formation par la recherche	C6 Stratégie et projet à cinq ans
A+	48%	45%	37%	53%	47%	43%
A	41%	48%	51%	32%	47%	45%
B	9%	5%	3%	5%	4%	10%
C	0%	0%	0%	2%	0%	1%
Non Noté	2%	2%	9%	8%	2%	1%



7 • Supervising bodies' general comments

UNIVERSITÉ PARIS DESCARTES

Vice Président du Conseil Scientifique

Paris le 09.04.2013

Vos ref : S2PUR140006303 -
Laboratoire de Neurophysiologie et
Nouvelles Microscopies - 0751721N

Monsieur Pierre GLAUDES
Directeur de la section des unités de recherche
Agence d'Evaluation de la Recherche et de
l'Enseignement Supérieur
20, rue Vivienne
75002 PARIS

Monsieur le Directeur

Je vous adresse mes remerciements pour la qualité du rapport d'évaluation fourni à l'issue de la visite du comité d'expertise concernant l'unité « Laboratoire de Neurophysiologie et Nouvelles Microscopies »

Vous trouverez ci-joint les réponses du Directeur de l'unité, Serge CHARPAK, auxquelles le Président et moi-même n'avons aucune remarque particulière à apporter.

Je vous prie d'agréer, Monsieur le Directeur, l'expression de ma considération distinguée.

Le Vice Président du Conseil Scientifique

Stefano Marullo, DM, DesSci

Université Paris Descartes, Vice-présidence du conseil scientifique, 12 rue de l'école de médecine, 75170 PARIS cedex 06
Téléphone: 01 76 53 17 45; Courriel: stefano.marullo@parisdescartes.fr

Laboratory of Neurophysiology and New Microscopies

We greatly appreciate the general analysis of our unit project, however we would like to comment the report on two minor grounds, i) one concerning the wish of PhD students and postdocs to participate to a higher number of international conferences and ii) another concerning the risky aspect of team 3 projects.

i) Participation to international conferences
As indicated to the committee after the visit, the questions had not been fully understood by our students and postdocs. They did not mention the Gordon, Jacques Monod and FENS and SFN conferences that they attended. During their thesis, all lab students must participate to at least 3 international conferences.

ii)Team 3

We thank the AERES committee for their comments on the successful accomplishments achieved by this team which emerged from the laboratory in 2010. We also appreciate the strong interest on the futur scientific program of this team that, we also believe, will constitute an outstanding and challenging work. We acknowledge, however, that some technical aspects of the program may appear risky. For this reason, we would like to respond in more details to the remarks of the committee, indicated in Bold:

In theme i), the first goal (a) is to investigate GABAergic input on NG2 cells with holographic photolysis of MNI-glutamate, which is certainly a promising approach to search for the (relatively few) synapses connecting both cell types. This paragraph is method-centered and lacks a clear scientific question/hypothesis, although developmental studies were mentioned in its heading.

We stated in the AERES manuscript and during the visit (January 2013) that our aim was to determine the identity of the interneurons providing GABAergic input to NG2 cells and to investigate the synaptic properties of unitary identified interneuron-NG2 cell connections in the developing somatosensory cortex. Although optical techniques have been successfully used in neurons to unravel neuronal connectivity, this kind of studies completely lacks for NG2 cells. Contrary to the committee's comment and the general belief that NG2 cells are poorly innervated, our preliminary data combining holographic photolysis and paired-recordings already indicate that NG2 cells in young mice are densely connected by different types of interneurons.

Viral approaches to knock down GABA receptor subunits are shortly mentioned. This might prove difficult, given the obvious problems with virus-based NG2 cell infections in vivo.

As mentioned in the AERES manuscript, we will use a dual approach in this part of the project, using either conditional knockout mice or lentivirus infections. During the oral presentation, we clearly explained that our current strategy was to generate a knockout mouse for the γ2 subunit of $GABA_A$ receptors, specifically in NG2 cells (and not to follow a lentiviral approach). In fact, we have already established a colony of γ2-flox knockout mouse that will be crossed with PDGFα-cre mice. This choice was motivated by our previous data, described in the result section of the AERES manuscript, demonstrating that the γ2 subunit in NG2 cells is a crucial component of synaptic $GABA_A$ receptors in NG2 cells.

In goal (b), it is intended to investigate interneuron-NG2 cell signalling after perinatal hypoxia (PH), to better understand the potential role of the neuronal input on glial proliferation and differentiation.
It is argued that the reduction of interneuron density will affect NG2 cell activity. This approach appears not completely convincing because PH may also affect NG2 cells through mechanisms other than altered GABAergic input. What is exactly meant by the term 'reacted NG2 cells' remains unclear.

Perinatal hypoxia (PH) is an animal model that induces an acute loss of pre-myelinating oligodendrocytes resulting in myelination defects. "Reactivated NG2 cells" is the term classically used to name endogenous NG2 cells that

increase their proliferation rate following hypoxic lesions (or any demyelinating lesion). This reactivation of NG2 cells obviously constitutes a complex process that depends on many extracellular and intracellular signals. However, if GABAergic synaptic inputs play a role in proliferation and/or differentiation, we expect that "reactivated NG2 cells" show a different GABAergic synaptic connectivity that correlates to the proliferation and/or differentiation process of these cells in PH lesions. We already have a bulk of unpublished data showing a close correlation between a transient loss of synaptic activity during the phase of active proliferation of NG2 cells in a model of lysolecithin-induced demyelination in the adult mouse *corpus callosum*.

It is not clear either who will perform the planned EM analyses.

It is clearly stated in the text that Brahim Nait Oumesmar (ICM, Paris) will perform "morphological analyses". This includes immunocytochemistry and electron microscopy.

Finally, (goal c) two-photon based Ca2+ imaging experiments are planned in vivo, in collaboration with the two others teams. The approach is well structured and addresses a very important open question. Since by now clear evidence for the existence of synaptic input-induced Ca2+ signals in NG2 cells is lacking, this part of the project is risky. Before planning to investigate presumed changes in activity-induced Ca2+ signalling in PH, the signals have to be identified under control conditions (which is still outstanding). Since the team did not start the experiments on PH yet and because the feasibility is not guaranteed, this part of the project is not put forward for the moment.

We fully agree with the AERES committee that this part of the project is ambitious since we want to search for Ca2+ signaling in NG2 cells *in vivo*. However, we clearly stated that we will first analyze Ca^{2+} responses in normal animals and then compare them to PH. This is certainly challenging, but it is realistic since it was recently demonstrated that Ca^{2+} transients in somata and processes of NG2 cells are evoked by electrical stimulation of nearby neurons when recorded with a two photon-microscope in acute hippocampal slices (Honsek et al., 2012 Hippocampus). To conclude, we agree that observing Ca^{2+} signaling *in vivo* in any condition will be an outstanding study.

Theme ii) addresses two questions: a) does glutamatergic synaptic onto NG2 cells in white matter impact proliferation and differentiation, and b) does it affect remyelination. In goal a) lentiviral infection is planned to knock down GRIP1, a protein that interacts with the GluR2 subunit which is most abundant in NG2 cells. The proposed loss of function effect will be investigated at different developmental stages at axonal-NG2 cell synapses, after injection of lentivirus-mediated shRNA. In addition, a gain of function approach is planned, using optogenetic lentiviral vectors. This is a very interesting project. However, no evidence is provided demonstrating the feasibility of lentiviral infection in vivo (cf. above). The collaborating group has published successful lentiviral transfection of OPCs in culture, but apparently not in vivo. In goal b), the group will investigate which alterations NG2 cells undergo in lysolecithin-induced demyelination.

We are aware that infection of NG2 cells will be lower *in vivo* than *in vitro*. For this reason, as mentioned during the oral presentation, our collaborated Brahim Nait Oumesmar (ICM, Paris) has produced lentivirus not only against GRIP1, but also against different subunits of AMPA receptors . He is presently testing them in culture to select the most efficient ones to be injected *in vivo*. If direct injection of these viruses *in vivo* does not work, we plan to use a second approach that consists in grafting transfected OPCs in the brain. The team of Nait Oumesmar is a leader of this type of techniques in the field of myelin (Maire et al., 2009, J Neurosci Res, Buchet et al., 2011, Brain).