11 Boutros, M., *et al.* (2000) Signaling specificity by Frizzled receptors in Drosophila. *Science* 288, 1825–1828
12 Willnow, T.E. *et al.* (1999) Lipoprotein receptors: new roles for ancient proteins. *Nat. Cell Biol.* 1, E157–E162
13 Trommsdorff, M. *et al.* (1999) Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97, 689–701
14 Wehrli, M. *et al.* (2000) arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* 407, 527–530
15 Pinson, K.I. *et al.* (2000) An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* 407, 535–538
16 Tamai, K. *et al.* (2000) LDL-receptor-related proteins in Wnt signal transduction. *Nature* 407, 530–535
17 Lin, X. and Perrimon, N. (1999) Dally cooperates with Drosophila Frizzled 2 to transduce Wingless signalling. *Nature* 400, 281–284
18 Tsuda, M. *et al.* (1999) The cell-surface proteoglycan Dally regulates Wingless signalling in Drosophila. *Nature* 400, 276–280

S.J. Pleasure
Neurodevelopmental Disorders Laboratory, Dept of Neurology, Room S256C, 513 Parnassus Avenue, University of California, San Francisco, CA 94143, USA.
e-mail: samuelp@itsa.ucsf.edu

Meeting Report

What you see is what you think – or is it?

Luc Pellerin, Nicola R. Sibson, Nouchine Hadjikhani and Fahmeed Hyder

Neuroenergetics: Relevance for Functional Brain Imaging. Held at Strasbourg, France; 10–12 October, 2000. Organizers: Richard S.J. Frackowiak, Pierre J. Magistretti and Robert G. Shulman. Proceedings of the meeting are free and will be available from the Human Frontier Science Program in June 2001 (www.hfsp.org).

Functional brain imaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), are now widely used in both research and clinical settings. In these studies, the changes in cerebral blood flow (CBF), glucose utilization or oxygenation levels measured by PET and fMRI have been taken as specific indicators of alterations in neuronal activity, and these signals have been used to map brain function. Paradoxically, however, relatively little is known about the mechanisms that link the processes of neural activity and metabolism with blood flow. In view of novel *in vitro* and *in vivo* data that could provide new insights into such issues, a workshop sponsored by the Human Frontier Science Program (HFSP) was recently held in Strasbourg.

For a long time it was imagined that thought process (and the accompanying changes in neuronal firing rates) requires very little additional energy above that needed for maintenance of resting ionic gradients within neurons. Several presentations at the conference were concerned with the energetics of synaptic transmission, and speakers addressed the question of the main energy-consuming processes in transmission of information within the CNS. Simon Laughlin (Cambridge, UK) established a global energy budget for the cortex, based on basic biophysical and biochemical data published in the literature. His calculations showed that ion fluxes associated with electrical signaling dominate energy consumption. Action-potential propagation and synaptic transmission impose equal energy costs that rise with spike rate. These conclusions agreed with data presented by Louis Sokoloff (NIH, Washington DC, USA), who showed that glucose utilization *in vivo* increases linearly with the degree of activation. Another important conclusion of this energy budget is that, under physiological conditions, 87% of total energy consumption is caused by signaling, whereas non-signaling (resting) energy expenditure represents only 13%. These estimations agree closely with measurements presented by Douglas Rothman in collaboration with Robert Shulman's group (Yale, New Haven, USA). They determined that glutamatergic transmission represented 80–90% of total energy cost, whereas non-signaling energy cost accounted for only 10–20%.

That glutamatergic neurotransmission dominates the energy budget should come as no surprise because ~80% of all neurons in the cortex are excitatory and ~90% of all synapses are glutamatergic.

A persistent dogma in brain energetics is that glucose utilization is restricted almost exclusively to neurons and is determined by the level of synaptic activity. Thus, a second theme of the meeting was the role of neuron–glia interactions in brain energy metabolism. Pierre Magistretti (Lausanne, Switzerland) presented evidence that uptake of glutamate into glia couples synaptic activity to energy metabolism. He showed that uptake of glutamate in astrocytes triggers aerobic glycolysis and lactate production, with a predicted consumption of one glucose for each glutamate molecule. Louis Sokoloff's results confirmed such conclusions, but also further emphasized the potential role of lactate as an energy substrate for neurons. The efficiency with which glial glutamate transporters clear glutamate was supported by the work of Craig Jahr (Portland, USA). Douglas Rothman also reported that glutamate–glutamine cycling, and hence astrocytic glutamate uptake, is a major metabolic pathway in the brain that is coupled to glucose oxidation in a close to 1:1 stoichiometry. This is intriguingly similar to the relationship between glutamate uptake and glucose utilization in astrocytes. Although a consensus seems to be emerging on the potential contribution of astrocytes to glucose utilization and activity at glutamatergic synapses, the exact nature and specific use of neuronal energy substrates is still open to debate. For example, Marianne Fillenz's (Oxford, UK) presentation supported the view that during activation, neurons would predominantly consume glucose, but after activation would turn to lactate produced by astrocytes as an additional fuel.

Most people that perform functional brain imaging techniques have so far assumed that changes in CBF, glucose utilization or oxygen consumption are each a precise reflection of neuronal activity. This view was first challenged by reports that changes in some of these parameters do not always match (the 'uncoupling paradox', see Ref. 1). Several speakers discussed the use of methods that measure changes in brain energy metabolism and whether these would directly or indirectly map neuronal activity. Kamil Ugurbil

 0166-2236/01/$ – see front matter © 2001 Elsevier Science Ltd. All rights reserved. PII: S0166-2236(00)01715-X

(Minneapolis, USA) addressed the issue of using the spatial specificity of the blood-oxygenation-level-dependent (BOLD) signal during fMRI at high magnetic fields to map neuronal activity and the possibility of calibrating fMRI to determine oxygen consumption. Using PET, Marcus Raichle (St Louis, USA) reported that although changes in blood flow are accompanied by changes in glucose utilization of approximately equal magnitude and spatial extent, they far exceed changes in oxygen consumption. This was confirmed, at least to some extent, by Albert Gjedde (Aarhus, Denmark), with the caveat that the relationship between blood flow and oxygen consumption might depend on whether the stimulus is physiological or non-physiological. From his work on the cerebellar cortex, Martin Lauritzen (Copenhagen, Denmark) suggested that neurovascular coupling might not be as straightforward as previously assumed and is possibly not valid under all situations. Moreover, in some cases, changes in blood flow appear to be related to presynaptic activity but are independent of postsynaptic response. During discussions, the idea emerged that some of the observed discrepancies between blood flow, glucose utilization and oxygen consumption could arise from differences in the time course of measurement of each of these parameters. In addition, it was realized that an increase in blood flow might not be required to sustain the increase in metabolism observed during activation, raising a fundamental question about using blood flow as a reliable index of neuronal activity.

The major outcome of this meeting, however, is the realization that many controversies that have been lurking in the field for sometime (e.g. high energy cost of signaling, role of non-neuronal cells in regulation of energy metabolism, relationship between neuronal activity and neurotransmitter flux, uncoupling phenomenon between imaging parameters) can now potentially be resolved, simply because they can be formulated in much better terms. This is the benefit of bringing together people with different expertise to address such fundamental problems – the major objective of these HFSP workshops. But, even if the broad contours of the mechanisms that give rise to brain-imaging signals are beginning to be unraveled, we still need to take a closer look to distinguish the details.

Acknowledgements
We would like to thank Maria Adams for help compiling this report.

Reference

1 Fox, P.T. and Raichle, M.E. (1986) Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc. Natl. Acad. Sci. U. S. A.* 83, 1140–1144

Luc Pellerin*
Institut de Physiologie, Université de Lausanne, Switzerland.
*e-mail: luc.pellerin@iphysiol.unil.ch

Nicola R. Sibson
Dept of Biochemistry, University of Oxford, UK

Nouchine Hadjikhani
Dept of Radiology, Massachussets General Hospital, Charlestown, MA, USA.

Fahmeed Hyder
Diagnostic Radiology, Yale University School of Medicine, New Haven, CT, USA.

Recent advances in neural stem cell technologies

Maeve A. Caldwell

American Society of Neuroscience. Held at New Orleans, LA, USA. 4–9 November, 2000.

One of the major attractions at the American Society for Neuroscience meeting was the stem cell symposium, held on November 4th and sponsored by Promega Neurosciences. The large attendance at this symposium and the surge in publications in neural stem cell biology over the past ten years attests to the great interest in this fast-expanding field of neuroscience. Many of the world leaders in this 'hot' area were present and gave a flavour of their work in this field, with the keynote speaker being Fred Gage (San Diego, USA). The symposium was opened by David Kaplan (Montreal, Canada) who acted as moderator for the session.

Expanding neural precursor cells

There are two methods of growing neural stem cells. The first method involves growing precursor cells on a substrate-coated surface, on which the cells expand as a monolayer in a two-dimensional (2-D) environment[1]. The second method involves growing cells in a three-dimensional (3-D) environment on non-coated plastic as clusters of cells termed neurospheres[2] (Fig. 1a). The life span of human precursor cells is critically dependent on maintaining cell-to-cell contact during proliferation. In addition, the length of time human neurospheres can be expanded in culture can be potentiated with the addition of leukaemia inhibitory factor (LIF). This results in 90 population doublings compared with 40 in the absence of this factor. Human cells contain low levels of telomerase[3]. Every time a cell divides, the telomeres at the end of chromosomes are eroded and hence they become shorter, and once they have eroded to a certain length the cell senesces. As explained by Clive Svendsen (Cambridge, UK) LIF works by activating telomerase and therefore allows these cells to more than double their life span. The potential of these expanded cells is an important issue. If human cells are sorted into two populations on the basis of size (large and small) using fluorescent-activated cell sorting (FACS), the large cells stain positive for glial fibrillary acidic protein (GFAP) (indicative of astrocytes) and negative for TuJ1 (indicative of neurons), whereas the small cells stain positive for TuJ1 and negative for GFAP. However, if these populations are re-aggregated so that they form new spheres, only the large cells are able to continue proliferating, and upon differentiation can give rise to neurons. This suggests that large cells are bi-potent: they can divide to give rise both to neurones and to astrocytes *in vitro*
(C. Svendsen). Fred Gage presented work showing that *in vivo* dividing cells are found in dense clusters associated with the vasculature. These clusters consist of

 0166-2236/01/$ – see front matter © 2001 Elsevier Science Ltd. All rights reserved. PII: S0166-2236(00)01725-2