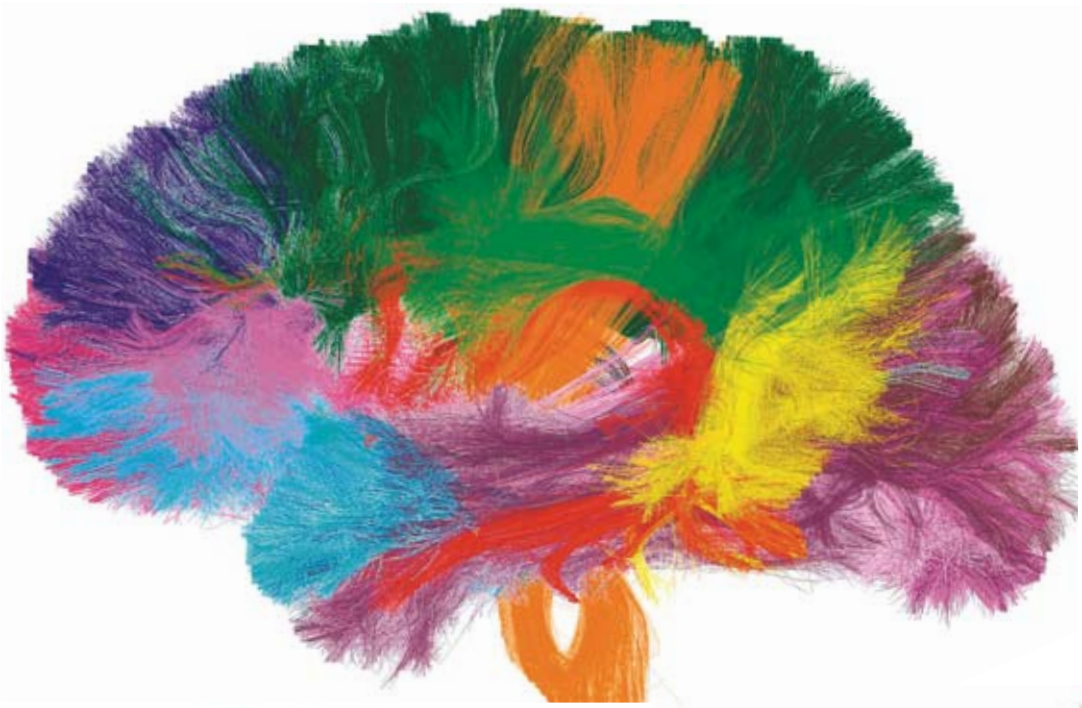
No. 62
Autumn 2014

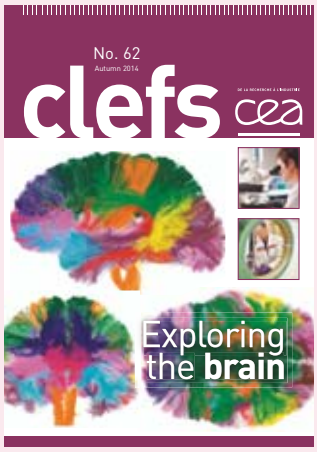
clefs

DE LA RECHERCHE À L'INDUSTRIE

cea



Exploring the **brain**



Clefs CEA No. 62 – AUTUMN 2014

Main cover picture
Representations of 38 bundles of cerebral white matter (one colour per bundle) taken from the first *in vivo* atlas of human brain connections. Each one is an assembly of images acquired from 78 subjects in the CONNECT/Archi neuroimaging database, which was set up at NeuroSpin, using cross-sections that are sagittal (vertical; the sections scan the subject from left to right), coronal (vertical; the sections scan the subject from front to back) and transversal (horizontal).
D. Duclap, B. Schmitt, A. Lebois, P. Guevara, D. Le Bihan, J.-F. Mangin, C. Poupon/CEA-NeuroSpin

Inset
top: MIRCen histology platform. Brain slices marked by different specific indicators are being examined with a microscope.
C. Dupont/CEA

bottom: Preparation of a radiopharmaceutical in a shielded hot cell in the Frédéric Joliot Hospital Service (SHFJ).
P.-F. Grosjean/CEA

Pictogram on inside pages
Alex Mit/Shutterstock.com

Review published by CEA
Communication Division
Bâtiment Siège
91191 Gif-sur-Yvette Cedex (France)
Phone: + 33 (0)1 64 50 10 00

Executive publisher
Xavier Clément

Editor in chief
Martine Trocellier
martine.trocellier@cea.fr

Contributor to the preparation of this issue
François Saint-Jalm

Scientific committee
Claire Abou, Bernard Bonin, Jean-Pierre Bruhat, Catherine Fillet, Étienne Klein, Gérard Sanchez, Régine Trébossen

Iconography
Florence Klotz

Production follow-up
Lucia Le Clech

Subscription
Subscription (printed French version) to *Clefs CEA* is free.
Requests for subscriptions should be addressed, preferably *via* Internet, by using the form available at the site: http://www.cea.fr
or by fax to: + 33 (0)1 64 50 20 01

Translated into English by
AéroTraduction

ISSN 1625-9718
Clefs CEA (Web)

Design of electronic media
Calathea - Paris
Phone: + 33 (0)1 43 38 16 16

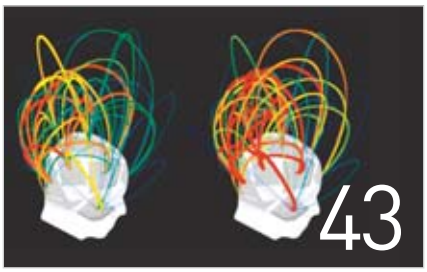
RCS Paris B 775 685 019
Head office: Bâtiment Le Ponant D, 25, rue Leblanc, 75015 Paris (France)

Exploring the brain

I. NON-INVASIVE EXPLORATION OF THE BRAIN

II. DEVELOPMENT, LEARNING AND PLASTICITY OF THE BRAIN

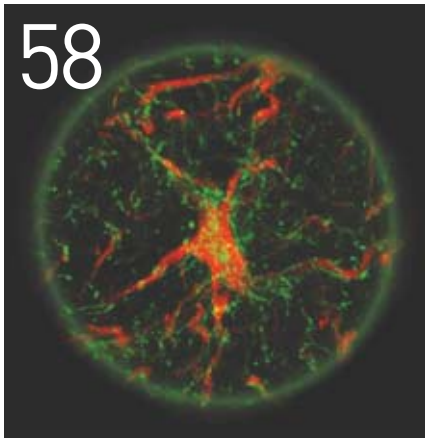
III. COGNITIVE ARCHITECTURE AND THE BRAIN

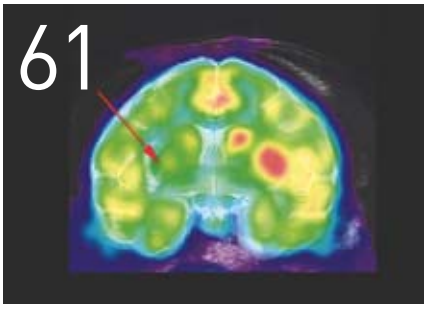


IV. MENTAL HEALTH AND VULNERABILITY

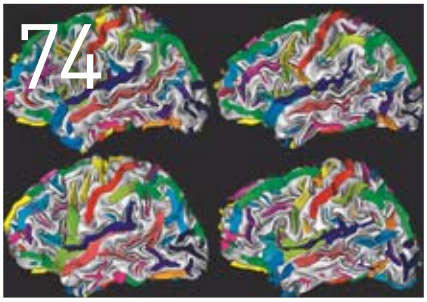
The articles of this chapter are not available

V. NEURODEGENERATIVE DISEASES





VI. IDENTIFYING BIOMARKERS FOR CEREBRAL DISORDERS



CEA, a major player in the field of research, development and innovation

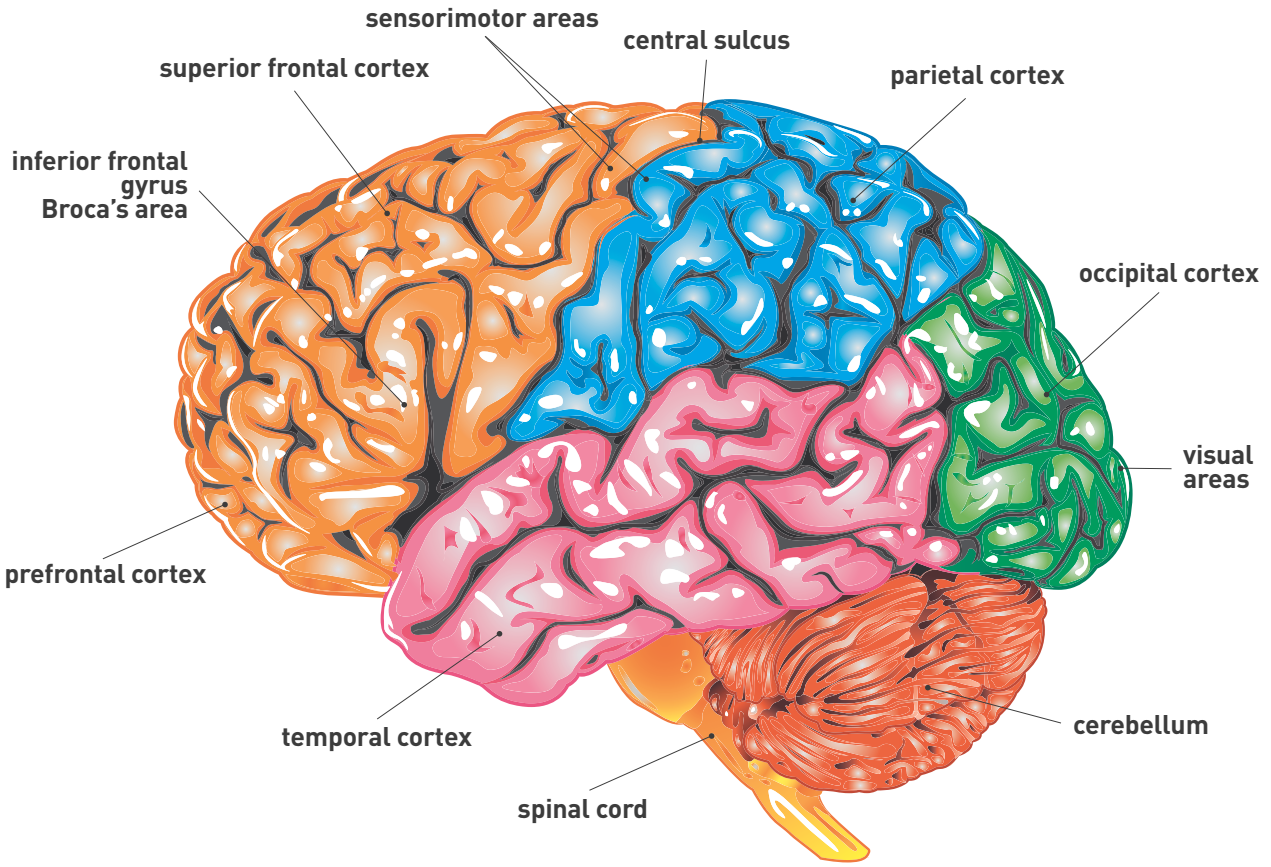
Standing as it does as a major player in the field of research, development and innovation, the French Alternative Energies and Atomic Energy Commission (CEA) is active in four main areas: low-carbon energies (nuclear and renewable), information technologies and health technologies, the design and operation of very large research infrastructures, and defense and global security. For each of these four main fields, CEA relies on first-rate fundamental research and offers support to industry.

CEA operates 10 research centres in France, distributed across the country. It is developing numerous partnerships with other research organizations, local authorities and universities. In this respect, CEA is a stakeholder in national alliances coordinating French research in the fields of energy (Ancre), life and health sciences (Aviesan), digital sciences and technologies (Allistene), environmental sciences (AllEnvi) and humanities and social sciences (Athena). Particular emphasis is also placed on education and information of the public at large, in order to promote the transfer of knowledge and foster the science-society debate.

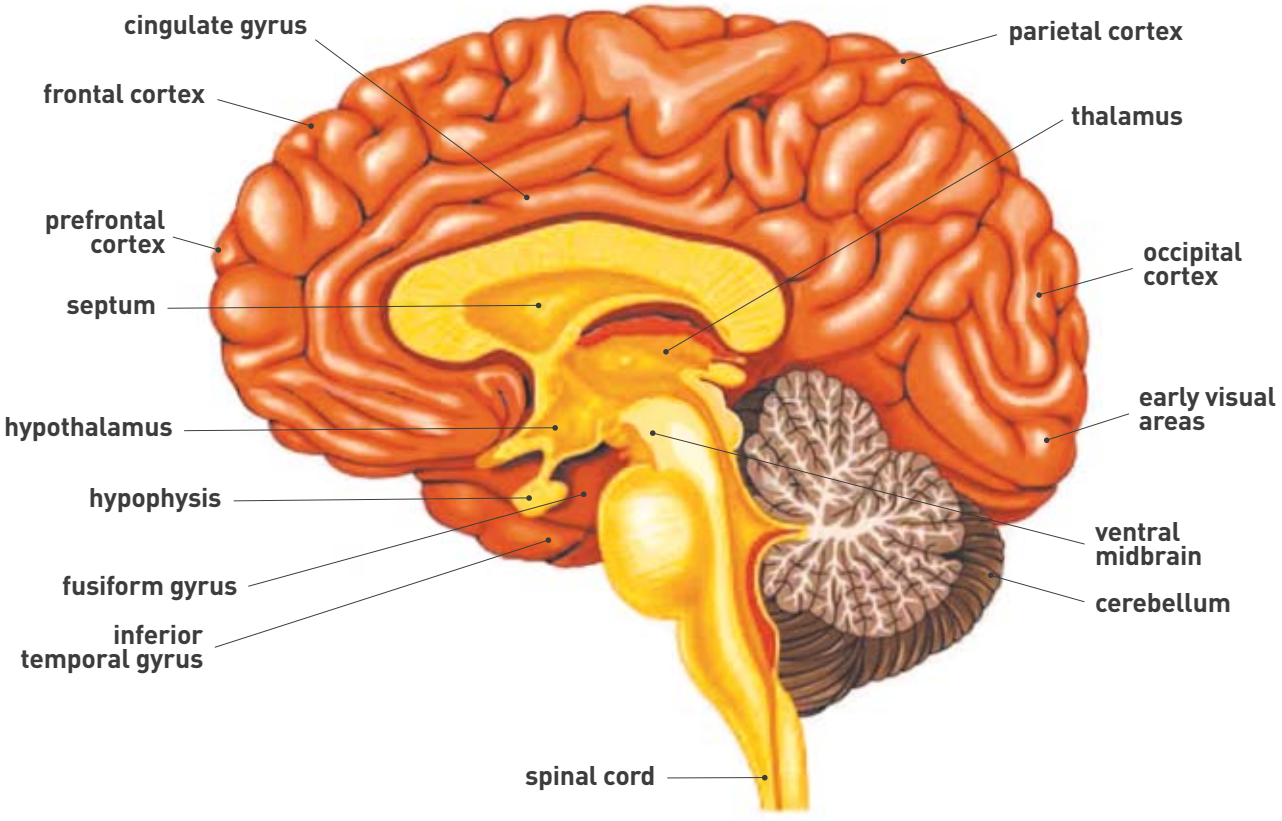
Widely recognized for its expertise in its areas of competence, CEA is fully engaged as a player in the European Research Area and is making its presence increasingly felt on the international scene.

Precision

On pages 2 and 4, illustrations of the human brain (whole and sections).



MedusArt/Shutterstock.com



Foreword

CEA/L. Godard

"Over 30 years after the first 2-tesla MRI, CEA is getting ready to welcome an 11.7-tesla MRI, which constitutes another world's first."

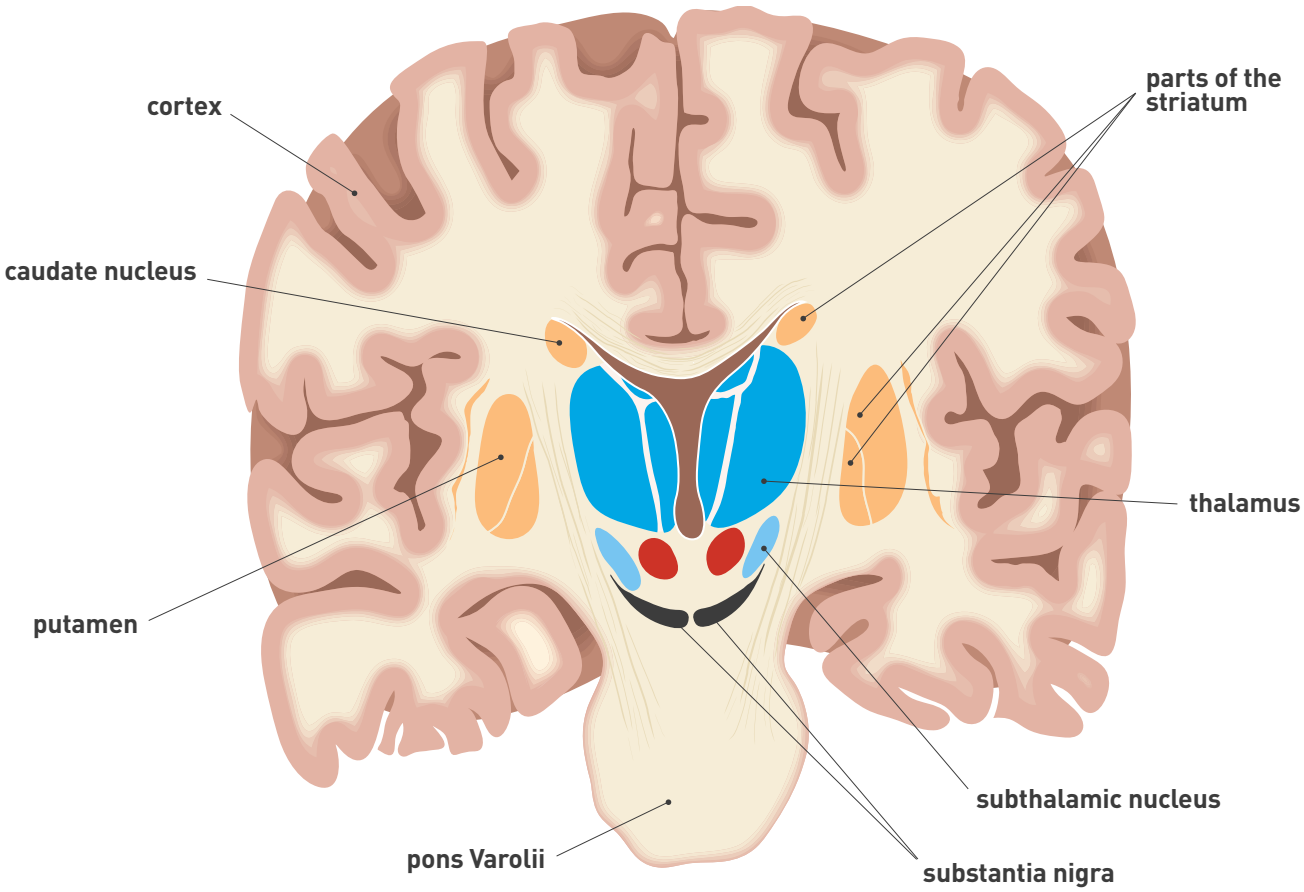
Carried by the strategic vision of great pioneers, CEA has been involved in brain research for over fifty years. This 62nd issue of *Clefs CEA* is the best occasion to come back on the latest advances of these works, undertaken most of the time in partnership with other research organizations such as Inserm (the French National Institute of Health and Medical Research), CNRS (the French National Centre for Scientific Research), Inria (the French National Institute for Research in Computer Science and Control), the *Collège de France*, and Paris-Sud University.

CEA's involvement in this wide field – which accounts for about 20% of research in biology in France – initially started with the use of artificial radioisotopes to explore the brain in action. The cradle of this research since 1958, the Frédéric Joliot Hospital Service (SHFJ) welcomed the first PET camera in France at the end of the 1970s and the first 2-tesla high-field MRI at the beginning of the 1980s. The latter was built thanks to CEA's know-how in intense fields. Three other large facilities dedicated to brain research span-off from SHFJ: Cyceron in Caen in 1985, NeuroSpin in Saclay in 2006, and MIRCen in Fontenay-aux-Roses in 2009. Each of these centres – all attached to the Institute of Biomedical Imaging (I2BM) of CEA's Life Sciences Division – hosts a high-level instrumental platform and neuroscience research teams jointly run with other organizations. They are capable of using *in vivo* imaging at the forefront of their research (including for clinical transfer) and at the same time of taking up new challenges for methodological developments in imaging. This stimulating dialogue between neuroscience, methodological research, and medical applications is also at the heart of the Clinatec biomedical research centre. This platform, launched in 2012 in Grenoble by CEA's Technological Research Division and its partners, is dedicated to the development of innovative treatments for brain diseases using micro-nano-technologies.

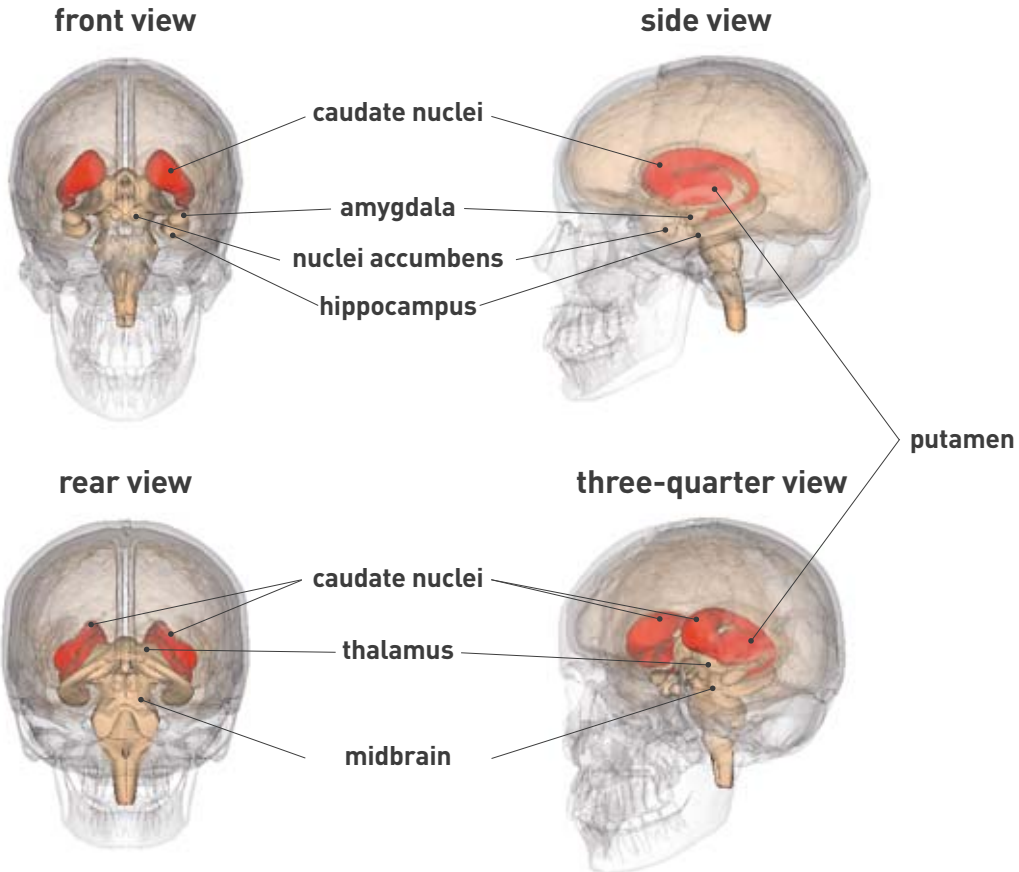
This long adventure – although swiftly drafted – is meant to continue. Over 30 years after the first 2-tesla MRI, CEA's biologists and physicists are getting ready to welcome an 11.7-tesla MRI, which constitutes another world's first. In the framework of the Paris-Saclay University Campus, CNRS and Paris-Sud University have also decided to gather their neuroscience research strengths within a new institute which will open in 2017 close to NeuroSpin.

Last but not least, CEA and its partners participate in the great European initiative referred to as the Human Brain Project meant to simulate the functioning of the human brain. It is thus always by relying on its internal assets, especially in the fields of physics, engineering, and large research instruments, but also on its national and international partnerships, that CEA intends to keep on contributing significantly to brain research.

> Gilles Bloch
Director of CEA Life Sciences Division



Blamb/Shutterstock.com



Images generated by Life Science Databases (LSDB)

The **complexity** of the nervous system: a challenge for **neuroscience**

Our consciousness of the world and of ourselves is unequivocal and coherent and is, to us, self-evident. However, the human brain, from which this consciousness originates, consists of a host of distinct elements. How is one to create order in and give meaning to this assembly of molecules, cells, **synapses** and other connections, networks and systems? How do these systems, which are constantly reconfiguring themselves, produce actions and representations, ideas and behaviours, thoughts and feelings and, finally, this consciousness?

The study of the **nervous system** has now entered the sciences of complexity era: there are apparently more synapses in a human brain than there are stars in 1,500 galaxies comparable to the Milky Way… The characteristics of the brain clearly reflect the concept of "emergence", this property of a system which cannot be deduced from the sum of the individual behaviours of its components. It is therefore necessary to observe, measure and experimentally manipulate the brain at all of its functional scales, from the smallest to the largest (from molecules to the whole brain) and from the fastest to the slowest (from one millisecond to periods of years).

This exploration of cerebral space and time constitutes a three-fold challenge. The first is instrumental and methodological: the extraordinary developments made in microscopy, cerebral imaging and neurophysiology have vastly improved our ability to observe and measure activity at all scales. Neuroscience is now open to physicists – from the fields of optics, magnetism, nuclear physics – as well as chemists, who are inventing new means of visualizing and modifying the activity of the nervous system. These methods are now supplementing **genetics**, physiology and molecular and cellular biology, which are just as imaginative, for example one need look only at intra-cellular calcium level indicators, or optogenetics.

The second challenge is experimental: it entails exploiting the progress made in instrumentation and developing appropriate animal models for the analysis of molecular or cellular networks, combined with behavioural observation. Numerous organisms – flies and worms, fish and birds, rodents and non-human primates – are used in the field of neuroscience to help us ask the right questions about the dynamic and multiscale organization of the brain's functions and how to measure and quantify them. The main mechanisms of the human pathologies, for example the neurodegenerative diseases, also need to be reproduced and modelled. The social cost of diseases of the nervous system is the highest of all pathologies, and therapeutic progress has been hampered by the lack of good animal models.

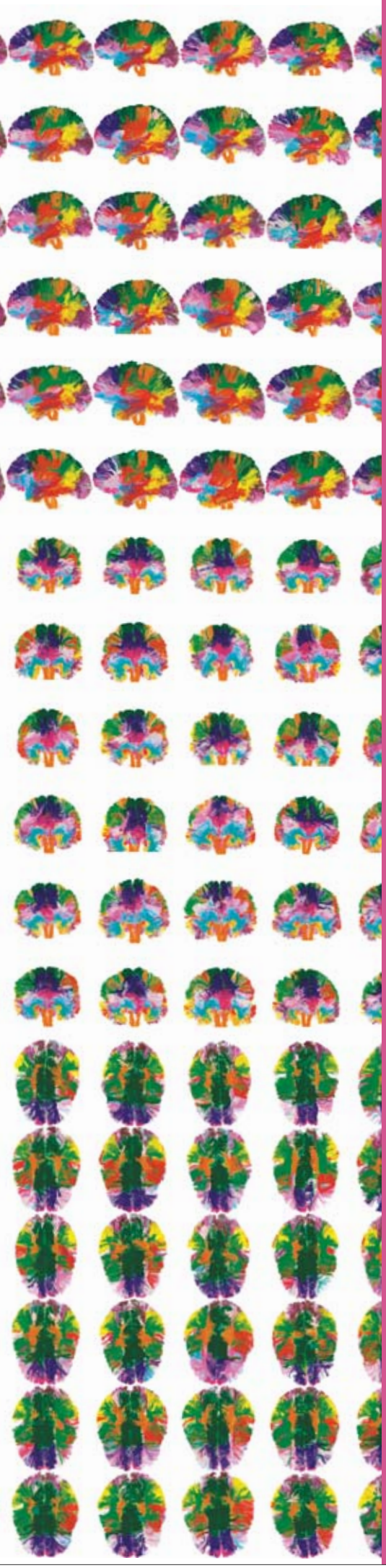
Naeblys/Shutterstock.com

Neuroscience focuses in particular on deciphering the functioning of our "thinking machine", from the molecule to the whole brain and in understanding the underlying physiopathological mechanisms of neurological diseases.

The third challenge is conceptual and theoretical: owing to its heterogeneity, its variability and its complexity, the nervous system poses serious problems in terms of processing and formalizing data, the volumes of which have become gigantic. These questions are familiar to physicists, computer scientists and mathematicians, but it is far from easy to bring together communities which are still relatively unused to working with each other. This is certainly the field in which the proactive approach of major projects such as the Human Brain Project can be most effective in meeting the challenge of reconstructing the working of the human brain at all its organizational levels.

In all of these fields, CEA teams are at the vanguard. Their contributions to the development of brain imaging instrumentation, to experimental and theoretical approaches, to **biomarkers** or to exploratory and therapeutic interventions, give them pride of place on the international stage.

> Philippe Vernier
Unit "Neurobiology and Development"
UPR 3294 CNRS, USC 1126 Inra
Institute of Neurobiology Alfred Fessard
CNRS Gif-sur-Yvette



Representation of fibres of white matter (1 bundle, 1 colour) in human brains. With the arrival of diffusion MRI, it is now possible to produce a detailed map of intra-cerebral connections in living subjects.

CEA

I. NON-INVASIVE EXPLORATION OF THE BRAIN

How can we observe the living brain, protected as it is by the skull? And will it one day be possible to "see thoughts"? It will only be possible to explore the living brain once physics has become a fully-fledged part of medicine. Everything starts with the measurement of the electrical activity of the brain, which gives an ElectroEncephaloGram (EEG). Brain imaging techniques proper were to appear during the last third of the 20th century. To obtain an image, it is necessary to find a physical signal to which the areas of the brain – which differ in their nature, their activity or their metabolism – react in different ways. Phenomena as diverse as X-rays, radioactivity or nuclear magnetic resonance were all to be make a contribution. Mathematics are also used in reconstructing the image and thus locating in the brain the origin of the signals collected externally. Other calculations are essential in order to reproduce the three-dimensional nature of the images or to reveal contrasts that are invisible on the original images.

The cerebral exploration techniques which use Magnetic Resonance Imaging (MRI) can currently be divided into three main categories. Anatomical imaging provides details, even very fine details, on the shape and volume of the various parts of a brain. For its part, functional imaging produces maps of brain activity in various conditions or in response to a variety of stimuli, while one of the purposes of diffusion MRI imaging is to produce maps of cerebral connectivity. Positron Emission Tomography (PET) gives access to metabolites and neurotransmitters, while the EEG and the MEG (MagnetoEncephaloGraphy) provide valuable information about the timing of events within the brain. All of these processes are complementary and are often jointly used. Much still needs to be done: continuing to improve temporal and spatial resolution, identifying new signals better able to reflect levels of neuronal activity, reducing the duration of examinations or artefacts in the images. It is only in this way that we will expand our understanding of a fascinating universe, our own, that of our brain.

> Denis Le Bihan
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Life Sciences Division
CEA Saclay Centre

Cerebral imaging: a never-ending story

From electroencephalography to diffusion tensor MRI, cerebral exploration and imaging techniques have constantly developed, with each innovation lifting the veil a little further to reveal more about this complex organ and how it functions. These methods are sources of fundamental knowledge about the healthy and sick brain and have become valuable clinical tools.

P.-F. Gros/CEA

The 7-tesla Magnetic Resonance Imaging (MRI) system at NeuroSpin.

The first internal image of a living body dates from 1895. It shows the bones of a hand – and a wedding ring. The German physicist Wilhelm Conrad Röntgen(1) used X-rays to photograph his wife's hand. The human body had suddenly become transparent! The brain, this soft tissue protected by the skull, had however remained beyond the reach of radiography and indeed all other non-invasive exploration techniques. This situation was to change in 1929, when the German neurologist Hans Berger(2) published the first human ElectroEncephaloGram (EEG). To achieve this, he had placed electrodes on the scalp of a patient in order to detect the electrical activity of his brain. This method was then improved by the British scientist Edgar Douglas Adrian(3) before becoming extensively used in the 1950s. Electroencephalography remains widely used today for functional exploration(4). This "simple" recording, which represents brain activity down to the nearest millisecond, is of valuable help in treating epilepsy, sleeping disorders or infectious diseases. However, even by using a large number of electrodes, it is impossible to precisely locate the source of the electrical signal in the brain. At best one can approximately situate the epileptic foci or, if there is a lack of activity, suspect the presence of a tumour or destroyed area.

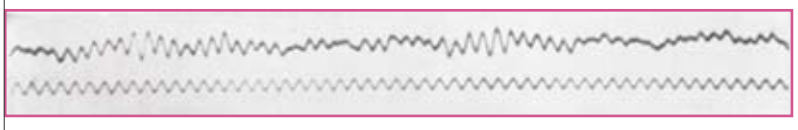
The physicists already know that any electrical current will create a corresponding magnetic field. So why not use this to observe brain activity? This is the basis of another global functional exploration technique: MagnetoEncephaloGraphy, or MEG (see box on *Magnetoencephalography*, p. 9). The physicist David Cohen at the University of Illinois (United States) captured the first magnetic signals from the brain in 1968. However, it was necessary to await the arrival of highly sensitive magnetometers, known as SQUIDs (Superconducting QUantum Interference Devices), to begin to be able to build satisfactory MEG devices in the early 1980s.

(1) Wilhelm Conrad Röntgen won the very first Nobel Prize in physics in 1901.

(2) Hans Berger built on the discovery of the brain's electrical activity by the British scientist and doctor Richard Caton in 1875.

(3) Edgar Douglas Adrian – with Charles Scott Sherrington – received the Nobel Prize in physiology or medicine in 1932.

(4) The EEG remains the reference method for determining brain death.



The first human electroencephalogram recorded in 1924 by the German neurologist Hans Berger and published in 1929.

P. Stroppa/CEA

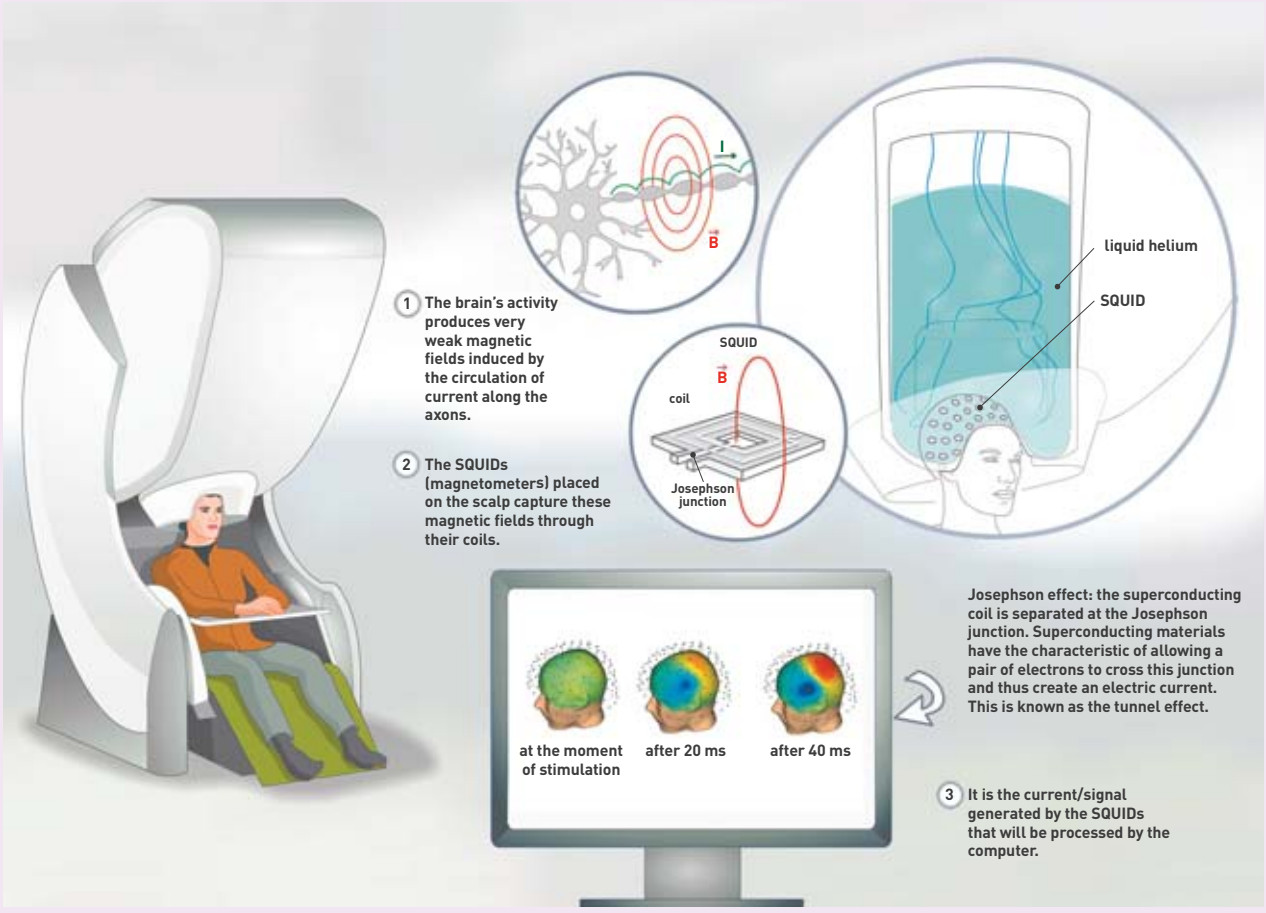
MagnetoEncephaloGraphy (MEG) installation at NeuroSpin. No fewer than 300 sensors continuously record the magnetic fields emitted by the currents circulating through the brain. By a method of reconstruction of the signal sources, the brain's activity is thus represented in space and time, giving access to the dynamics of information processing by the brain.

Magnetoencephalography

MagnetoEncephaloGraphy (MEG) records the magnetic activity of the brain. The origin of the electromagnetic signals is the way information circulates in the neural system: the neurons communicate with each other by means of local electrochemical changes which propagate along their membranes and accumulate in the synapses. Within the populations or assemblies of neurons, these changes produce electrical currents and therefore magnetic fields. Thus, on a large scale, the magnetic fields recorded with MEG come from the synchronous electrical activity of several tens of thousands of neurons. However, these fields are very weak, about one femtotesla (1 fT = 10^{-15} T), or a billion times smaller than the Earth's magnetic field, the value of which is close to 50 microteslas (1 µT = 10^{-6} T). MEG therefore uses extremely sensitive sensors known as SQUIDs (Superconducting QUantum Interference Devices), based on the superconducting properties of Josephson junctions. The MEG is placed in a chamber shielded and isolated by a mu-metal (alloy of nickel and iron) in order to filter out the surrounding electromagnetic noise.

The main quality of MEG lies in its ability to observe the cerebral processes to within a millisecond, non-invasively. The signals recorded provide pertinent temporal information about the brain's activity and thus enable a live recording to be taken in order to understand the neural "language". With the help of sophisticated inverse problem resolution methods, the origin of the MEG signals can be localized to within a few tens of millimetres, based on the anatomical MRI of the individual. MEG has proven its clinical worth in the screening of epileptic foci, but above all can be used to investigate the neural bases of the main human cognitive functions.

> Virginie van Wassenhove
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre



Principle of magnetoencephalography.

These then had to be installed in rooms carefully protected from all outside influences, including the magnetic field of the Earth itself... With a temporal resolution of the same order of magnitude as an EEG, an MEG gives better spatial data because the skull and the scalp do not deform the magnetic field (unlike the electrical field). The results must however be combined with anatomical data obtained using another method – anatomical MRI – in order to correctly locate the epileptic foci, neuronal degeneration or the consequences of traumas or ischemias. EEG and MEG are also methods commonly used to understand perception and human cognition.

Functional exploration of the healthy brain

How can one obtain a detailed image of a brain in the process of thinking? Or at least, how can one visualize the areas of the brain activated during the performance of a specific task? MEG and EEG give valuable information about brain activity over a period of time during complex cognitive tasks such as reading or mental imaging and, more generally, they enable time maps of cognitive functions to be produced. However, the cerebral sources of electromagnetic activity can only be mapped to

Positron emission tomography

This technique is based on a phenomenon that is well known in nuclear physics. The nucleus of certain stable elements, when bombarded by a proton, loses a neutron and becomes instable, or in other words **radioactive**. It regains stability by emitting a **positron** – the positive counterpart of the electron – which is annihilated almost immediately when it encounters an electron in the surrounding environment. This annihilation in turn triggers the simultaneous emission of two **gamma photons**(1) in diametrically opposed directions. To capture these photons, a ring of detectors must be placed around the medium to be observed, with only the pairs of photons arriving simultaneously on two opposed detectors(2) being selected. The positron source is necessarily on the axis linking the two receivers. With specific imaging computing methods, it is thus possible to reconstitute a slice of the emitting medium and then use a large number of slices to create a 3D image.

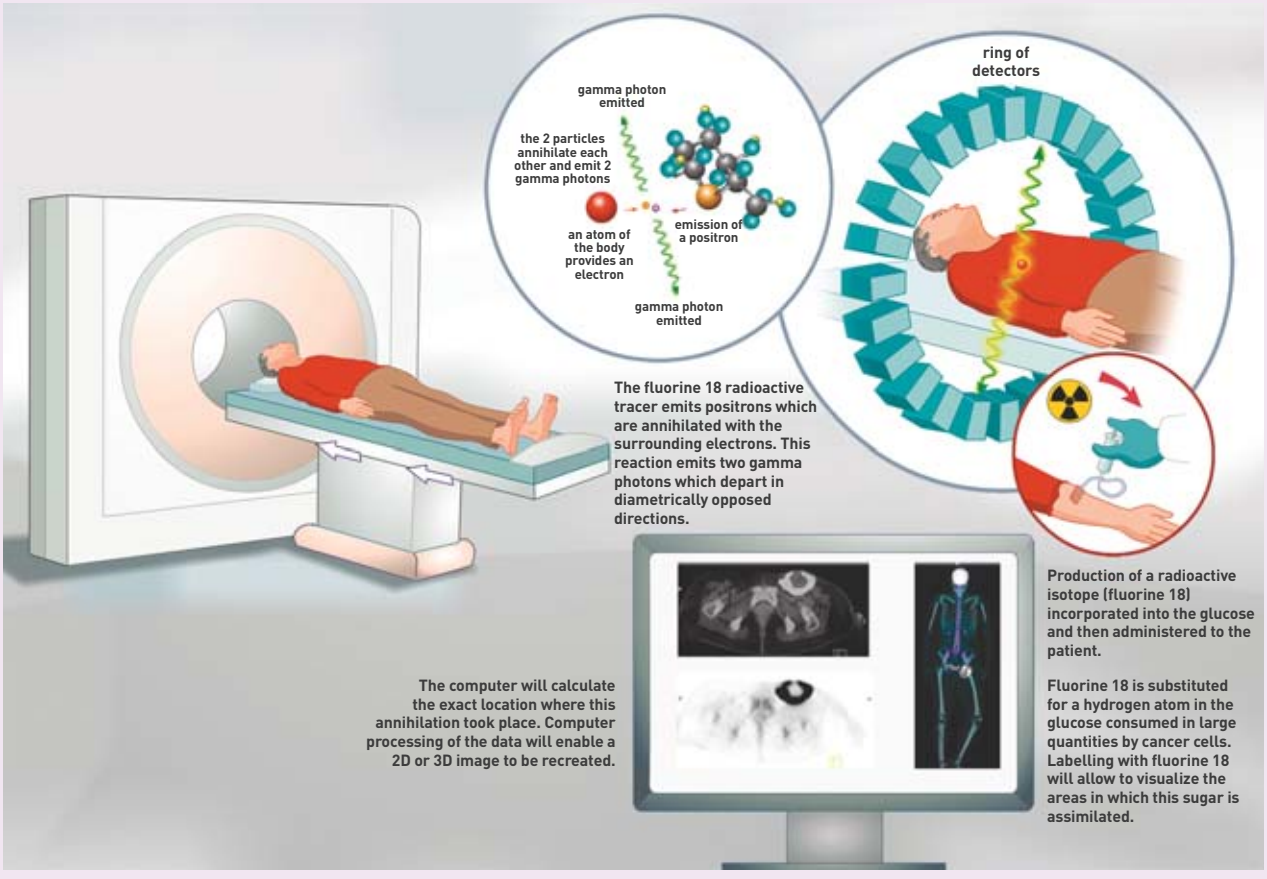
Living tissues do not spontaneously produce gamma photons and it is essential to place a positron-emitting element precisely at the spot one wishes to visualize, by injecting a molecule labelled with a radioactive atom and specifically chosen to target a biological phenomenon. The most widely used marker is an analogue of glucose comprising an atom of fluorine 18, 2-deoxy-2-[^{18}F]fluoro-D-glucose, or [^{18}F]-FDG. As glucose is the "fuel" for cells, this molecule is a good marker of the energy **metabolism**. The other major biological marker, more used by the neuroscientists, is quite simply water in which the oxygen atom has been bombarded, to produce oxygen 15.

Numerous PET markers are today available for exploring the biochemistry of brain cells and tumours in particular.

Given the short **radioactive half-life** of these elements (less than two hours for fluorine 18 and two minutes for oxygen 15), they have to be produced *in situ*. PET imaging units must therefore have a cyclotron (to bombard the stable nuclei with protons). With a **spatial resolution** of a few millimetres at best, PET is an excellent method for functional exploration but not for anatomical imaging. It is thus frequently coupled with MRI.

(1) These gamma photons have a characteristic energy level of 511 keV.

(2) In fact, they arrive almost at the same time because they are not necessarily emitted in the centre of the axis between the two receivers. The interest of this minute "time of flight" difference mainly concerns noise reduction in the reconstructed PET image.



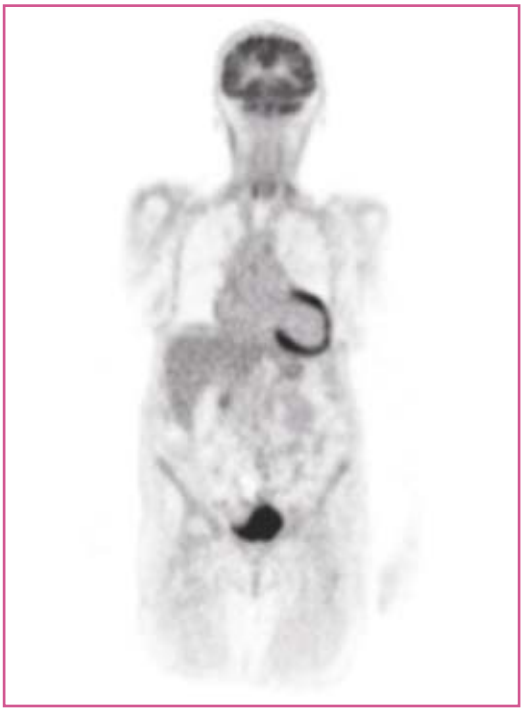
CEA/Corinne Beurtey

Principle of positron emission tomography.

within a few tens of millimetres. Higher anatomical **resolution** – to the detriment of temporal resolution – can then be obtained by Positron Emission Tomography, or PET (see box on *Positron emission tomography*, p. 10). The first application of a **positron** emitter for medical imaging goes back to 1951. The physicist Gordon L. Brownell and the neurosurgeon William H. Sweet of the Massachusetts General Hospital, in Boston (United States), visualized brain tumours by placing two detectors on either side of the patient's head. Even if the image was only planar, this system demonstrated the medical benefits to be gained from positron emission tomography. Tomography proper was born in the early 1970s, with the adoption of a 3-dimensional reconstruction algorithm developed by David A. Chesler, a member of Gordon L. Brownell's team. In order to measure the emissions simultaneously through 360°, multiple cameras are installed in a circle around the subject. The first appliance used on humans, Michael E. Phelps's PETT III (Positron Emission Transaxial Tomograph) at Washington University (Saint Louis, Missouri, United States), thus had 48 detectors with a diameter of 5 centimetres arranged in a hexagonal ring. Most of the PET appliances built since 1980 are based on this principle. Developments in detector instruments mainly involved an increase in their sensitivity and their miniaturization in order to improve **spatial resolution**. The most powerful PET appliances in use today comprise more than a hundred thousand millimetre sized detectors. For example, the HRRT (High Resolution Research Tomograph), which offers the best spatial resolution for brain imaging, comprises 119,808 detectors of 2 mm on a side, arranged in 104 octagonal rings.

The growth in PET starting in the 1980s, is closely linked to the development of **radioactive tracers** for numerous pharmacological targets, in particular for the study of the **central nervous system**. The first synthesis in Brookhaven National Laboratory (Long Island, New York, United States) of a traced derivative of glucose, [^{18}F]-FDG, in 1976, had a major impact on the development of PET (see box on *Positron emission tomography*, p. 10). It allows *in vivo* measurement of the regional cerebral **metabolism** of glucose. However, another **clinical** application became available in the late 1990s: the detection of malignant tumours. Whole-body PET examinations using [^{18}F]-FDG were to prove extremely useful for diagnosing and therapeutic monitoring in **oncology**. Thus in the 2000s, PET became widely used in nuclear medicine units in hospitals. The need to combine anatomical localization with the tumours detected on the images led the Americans David W. Townsend and Ronald Nutt to develop a first device associating PET with an X-ray scanner[5] in 1998. Since then, all PET appliances marketed have been combined with this anatomical imaging method.

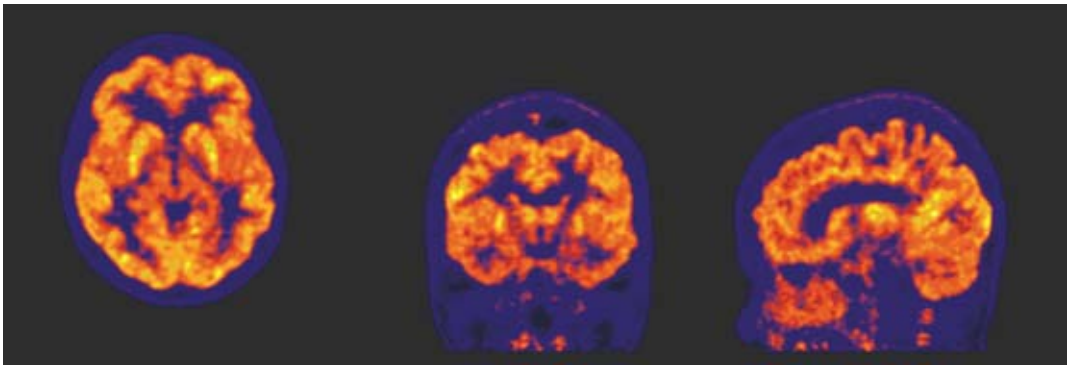
For the neuroscientists, PET however represents something else: for about fifteen years, it was to be the only functional imaging technique usable on the healthy brain. Like all the subsequent functional cerebral exploration methods, PET was based on a hypothesis formulated in 1890 by the British scientists Charles Smart Roy and Charles Scott Sherrington[3] and then confirmed by neurosurgeons: blood flow



CEA/SHFJ

Study of the regional metabolism of glucose in Positron Emission Tomography (PET) carried out for a suspected cancer. The images show areas of intense glucose metabolism (the heart, the brain, the muscles, the liver). This metabolism is physiological. No area of pathological hypermetabolism is evident on this image. This PET examination is carried out for cancer workups.

(5) In fact, any device which "scans" a sample to look for a physical signal is a scanner. MRI devices are therefore also scanners.



CEA/SHFJ

Brain metabolism of glucose in a healthy subject. The images are acquired on a Positron Emission Tomography (PET) system with very high spatial resolution capable of visualizing the metabolism in the **gyri** of the cerebral cortex.

increases in the activated regions of the brain. This phenomenon, as yet poorly explained, is called "neurovascular coupling". In order to visualize this using PET, the patient is injected with water labelled with oxygen 15, which enables the blood flow to be monitored. Owing to neurovascular coupling, the areas of the brain activated during performance of a task or reception of a stimulus became visible through the increased flow of the labelled water. Naturally one must first have taken an image of the brain "at rest", in order to show up the differences, and have precisely defined the task in order to avoid stray activations. In this respect, it was PET that laid the foundations of functional neuro-imaging and with which the cognitive specialists built up their methods and protocols, or in other words, their culture. Its limitations – apart from having to use a cyclotron – are linked to the need to inject a **radioactive** product, which rules out repeated examinations and use on infants or pregnant women. Furthermore, the very short **half-life** (2 minutes) of oxygen 15 is a serious constraint for functional exploration protocols. PET is today the most effective technique for molecular imaging[6] of the brain. Thanks to a wide diversity of radioactive tracers, it is extensively used in clinical research, to study complementary aspects of the various **neurotransmission systems** (**dopaminergic**, **serotonergic** transmission, etc.), which play an essential role in the transmission of information between the **neurons**.

The development of anatomical imaging

The first real revolution in anatomical medical imaging came about in 1972 with the arrival of X-ray Computed Tomography (X-ray CT), better known to the general public under the name scanner. The scanner was developed by the British engineer Godfrey Newbold Hounsfield on the basis of work done by the American physicist Allan MacLeod Cormack[7], and uses X-rays. However, two key innovations made CT different from conventional radiography. First of all, the photographic plate gave way to more sensitive sensors, giving up to 2,000 shades of grey instead of the traditional 4 corresponding to bones, water, fat and air. It was now possible to distinguish between different tissues. Above all, computer processing power was also able to reconstitute images in two and then three dimensions from numerous axial measurements[8]. This use of computing was to become omnipresent in medical imaging.

In just ten years, CT became standard equipment in hospitals, but the presence of the skull, which is opaque to X-rays, was a major obstacle to its use in exploration of the brain. By injecting a contrast medium (often iodine based) into the patient's blood, the doctors could however observe cerebral vascularisation and identify **aneurysms**, **angiomas**, or even abnormally vascularised regions indicating the possible presence of a tumour. Despite that, CT was not really suitable for detailed cerebral imaging.

MRI enters the scene

In 1973, the American chemist Paul Christian Lauterbur[9] proposed a means of making use of the nuclear magnetic resonance phenomenon (see box on *Magnetic resonance imaging*, p. 14) to produce anatomical images. Magnetic Resonance Imaging (MRI) was born… even if its inventor originally named it "zeugmatography"! In 1977, the British physicist Peter Mansfield[9] introduced the echo-planar imaging technique, which considerably sped up the image acquisition process. MRI was now "mature".

This technique uses the signals emitted by the nuclei of the hydrogen atoms present in the body, water molecules in particular. In other words, unlike computed tomography, MRI does not primarily "see" the bones, but rather the soft tissues. It is therefore the ideal instrument for exploring the brain. The contrasts between different areas of the brain, for example between **grey** and **white matter**, are not however based on their hydrogen content – which is relatively uniform – so much as a parameter called the relaxation time (see box on *Magnetic resonance imaging*, p. 14). It was the American physician Raymond Vahan Damadian who introduced this principle in the early 1970s. He was then looking to distinguish between cancerous and healthy tissues, but by adjusting the contrast it was possible to see many other things with magnetic resonance.

"Conventional" MRI today offers a spatial resolution of a fraction of a millimetre, using very low energy levels and without the slightest radioactivity. It can therefore be used repeatedly and is perfectly suitable for infants and pregnant women. The only known drawbacks are the intense magnetic field which rules out the use of an object that can be magnetized or is sensitive to a magnetic field (pacemaker, etc.), and the considerable noise made by the system in operation, which requires the use of ear defenders. It has nonetheless become the instrument of choice

P.-F. Grosjean/CEA

Images of the human brain acquired with the 3-tesla MRI system at NeuroSpin.

(6) Molecular imaging is based on the use of a tracer incorporated into a cellular biochemical process, the fate of which is identified thanks to labelling specific to the imaging technique.

(7) Godfrey Newbold Hounsfield and Allan MacLeod Cormack received the Nobel Prize in physiology or medicine in 1979.

(8) This reconstruction is based on a 1917 theorem by the Austrian mathematician Johann Radon.

(9) Paul Christian Lauterbur and Peter Mansfield received the Nobel Prize in physiology or medicine in 2003.

for anatomical imaging of the brain. Functional exploration was however to remain the preserve of PET… until the appearance of a new form of MRI, an imaging technique that was to prove particularly versatile.

Shedding light on the brain

Both PET and MRI require the use of costly installations. This is why a relatively inexpensive, non-invasive, silent, rapid and even portable technique has created its own niche: optical imaging (see box on *Optical imaging*, p. 13). The idea of using light to scan the interior of the human body goes back several centuries. In 1831 the British physician Richard Bright reported the case of a hydrocephalic patient whose skull he could observe against the light by means of a candle placed behind the head, with the examination being carried out otherwise in complete darkness. After other attempts consisting in illuminating an organ by candlelight or daylight and observing the light coming through with the naked eye, studies were conducted in the 1930s to try to detect breast tumours. This involved illuminating the breast with a lamp and examining its projected shadow. However, the results were not particularly reliable and doubt was cast on the clinical benefits of optical imaging. Light only penetrates biological tissues to a depth of a few tens of micrometres, so for a long time, the diffusion of light by the tissues only enabled the optical properties on the surface to be measured, in the same way as microscope systems.

The optical study of biological tissues therefore developed very little until 1977, when the American researcher Frans F. Jöbsis-vanderVliet from Duke University (Durham, North Carolina) demonstrated that infrared light can pass through thick biological samples, including the brain, to provide useful information. Optical imaging then developed rapidly and its applications include the detection of brain haemorrhages and damage to the white matter, as well as exploration both functional (variations in blood concentrations on the surface of the brain according to cerebral activity) and metabolic.

MRI becomes functional

In the early 1990s, PET's unchallenged domination of the functional exploration of the brain was threatened by MRI. The first alert, in 1991, was attributable to John Belliveau, of the Massachusetts General Hospital, in Boston (United States). His method involved the injection of a gadolinium-based contrast agent into the patient's blood. Owing to the phenomenon of neurovascular coupling, the activated areas of the brain then showed up on the image because their blood flow increases. The method was quickly abandoned because, in 1992, three teams simultaneously published articles presenting functional MRI as we know it today. This was based on an observation made on rats in 1990 by the Japanese physicist Seiji Ogawa, who at the time was working at Bell laboratories (Murray Hill, United States): blood vessels become more apparent in MRI when the animal is deprived of oxygen. The oxygenated

Optical imaging

Cerebral optical imaging utilizes light in the near infrared range (between 700 and 900 nanometres), capable of passing through considerable thicknesses of biological tissue. It exists in various forms and with various names: Near InfraRed Spectroscopy (NIRS), Diffuse Optical Topography and Tomography (DOT) or Near InfraRed Imaging (NIRI). The principle however remains the same: infrared light illuminates a point on the skull and is then detected as it exits at a distance of a few millimetres. The absorption spectra of various absorbent molecules (chromophores) encountered along the pathway, such as oxyhaemoglobin (HbO_2) and deoxyhaemoglobin (Hb), are analysed in order to determine their respective concentration and distribution, along with a total blood volume. By multiplying the number of infrared light sources and detectors, it is possible to quantify other chromophores, such as cytochrome oxydase, which is indicative of metabolic activity. Finally, it has also been shown that cerebral activity could be linked to a variation in blood flow and oxygenation in the active areas of the brain, although a number of studies are still in progress on this subject.

Depending on the target application, several acquisition techniques are possible (continuous wave, frequency domain, time resolved) each with its advantages and drawbacks in terms of information extracted, depth reached (a few centimetres), spatial resolution (about 1 cm) and temporal resolution (a few milliseconds to several minutes).

The geometry adopted for the infrared light sources and detectors gives access to local point contrast measurements or to 2D/3D maps of optical parameters in the brain.

Topography, which is the reconstruction of a map of blood concentrations in a slice of tissues (< 2 cm) on the surface of the brain, was used in children as of the 2000s, to observe the response of the cortex to a stimulus (functional activation). In adults, the hemodynamic response to various stimuli and the relationship between the consumption of oxygen and the neurovascular response have been the subject of research since the late 1990s. A more recent technique, which appeared in the late 2000s, DCS (Diffuse Correlation Spectroscopy), completes these hemodynamic measurements by non-invasive measurement of the blood flow in the cortex.

Tomography, a volume reconstruction of the optical parameters at depth (complete 3D volume), has been in use since the 2000s in newborns, in order to detect pathologies (haemorrhages, leukomalacia – lesions of the white matter –, tumours) or to monitor development of the infant.

Optical imaging has thus been used in neuroscience for about twenty years now, for pre-clinical or clinical studies applied to adults, children and premature infants. It is non-invasive, portable, robust and relatively inexpensive and offers an interesting approach for visualizing brain activation or searching for pathologies.

> **Anne Planat-Chrétien, Vincent Poher, Jean-Marc Dinten** and **Isabelle Texier**
Leti Institute (Laboratory of Electronics and Information Technologies)
Technological Research Division
CEA Grenoble Centre

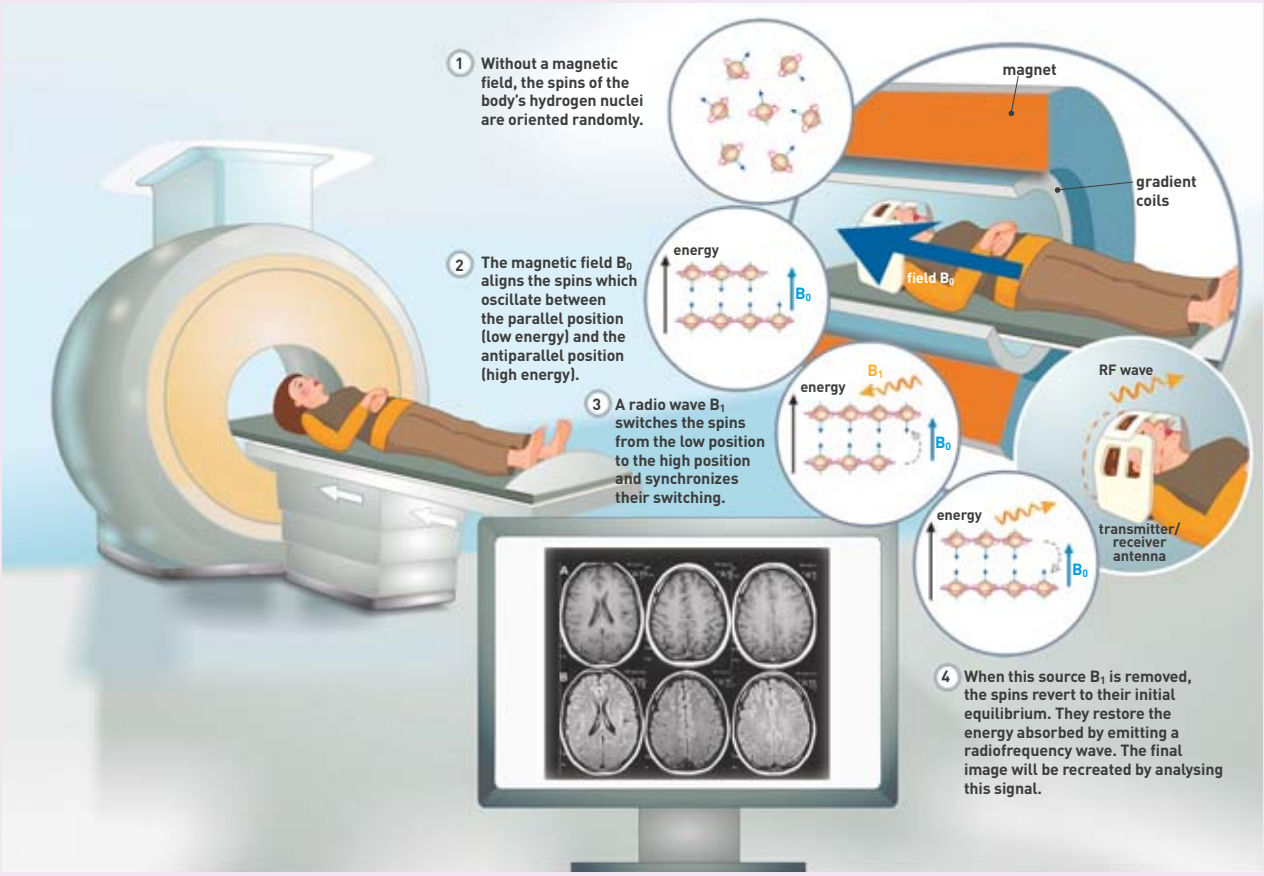
(oxy) or deoxygenated (deoxy) forms of haemoglobin do not have the same magnetic properties and this modifies the MRI signal of the neighbouring water molecules.
Does activation of an area of the brain modify the local level of blood oxygenation and can this be visualized with MRI? The answer to both questions is yes, as was shown by the three teams – that of Seiji Ogawa with the University of Minnesota in Minneapolis, that of John Belliveau and that of James S. Hyde at the Medical College of Wisconsin – with a classic response test to a visual stimulus, in this case a black and white checkerboard. Functional MRI, also known as BOLD (Blood-Oxygen-Level Dependent), had arrived. In reality, the activated

Magnetic resonance imaging

In 1946, two physicists, Félix Bloch from Switzerland and Edward Mills Purcell from the United States[1], discovered the phenomenon of Nuclear Magnetic Resonance (NMR). The nuclei of certain atoms, including that of hydrogen (proton ^1H), possess a magnetic moment associated with their spin. This aligns with the direction of a strong ambient magnetic field, rather in the same way as the needle of a compass, but is capable of adopting one or other of the two directions (parallel or antiparallel) owing to thermal agitation. If we then send a radio wave with a frequency specific to the element (42.6 MHz per tesla[2] for hydrogen for example), the spins parallel to the field absorb energy and go to the antiparallel position. At the same time, the spins synchronize their consecutive changes between the two positions – parallel (low energy) and antiparallel (high energy) – and the protons emit a wave of the same frequency, which can be captured with an antenna. After a short "relaxation time", this wave disappears and the magnetic moments return to their equilibrium alignments.
So how do we turn this into images? MRI is based on the fact that the resonance frequency of a given nucleus depends on the ambient magnetic field. By varying this field spatially, it is then possible to identify the origin of the signals retransmitted. When repeated in different directions, this operation is able to reconstruct 2D and even 3D images using conventional tomography methods. Medical MRI primarily focuses on the nucleus of hydrogen, a component of the water molecule present in most tissues, especially the brain.
An MRI system thus consists of a large superconducting electromagnet which outputs an intense, but fixed magnetic field, onto which a spatially-variable weaker field is superimposed by means of three conventional copper coils called the gradient coils, each oriented along one of the three spatial axes. Radio-wave transmission and reception antennas complete the set-up. The intensity of the signal, and therefore the quality of the images, increases with the magnetic field, hence the use of intense fixed fields, of about 3 T in clinical routines and far higher for certain research instruments. MRI is a non-invasive method offering excellent spatial resolution and can be used in a variety of modes: anatomical, functional or diffusion.

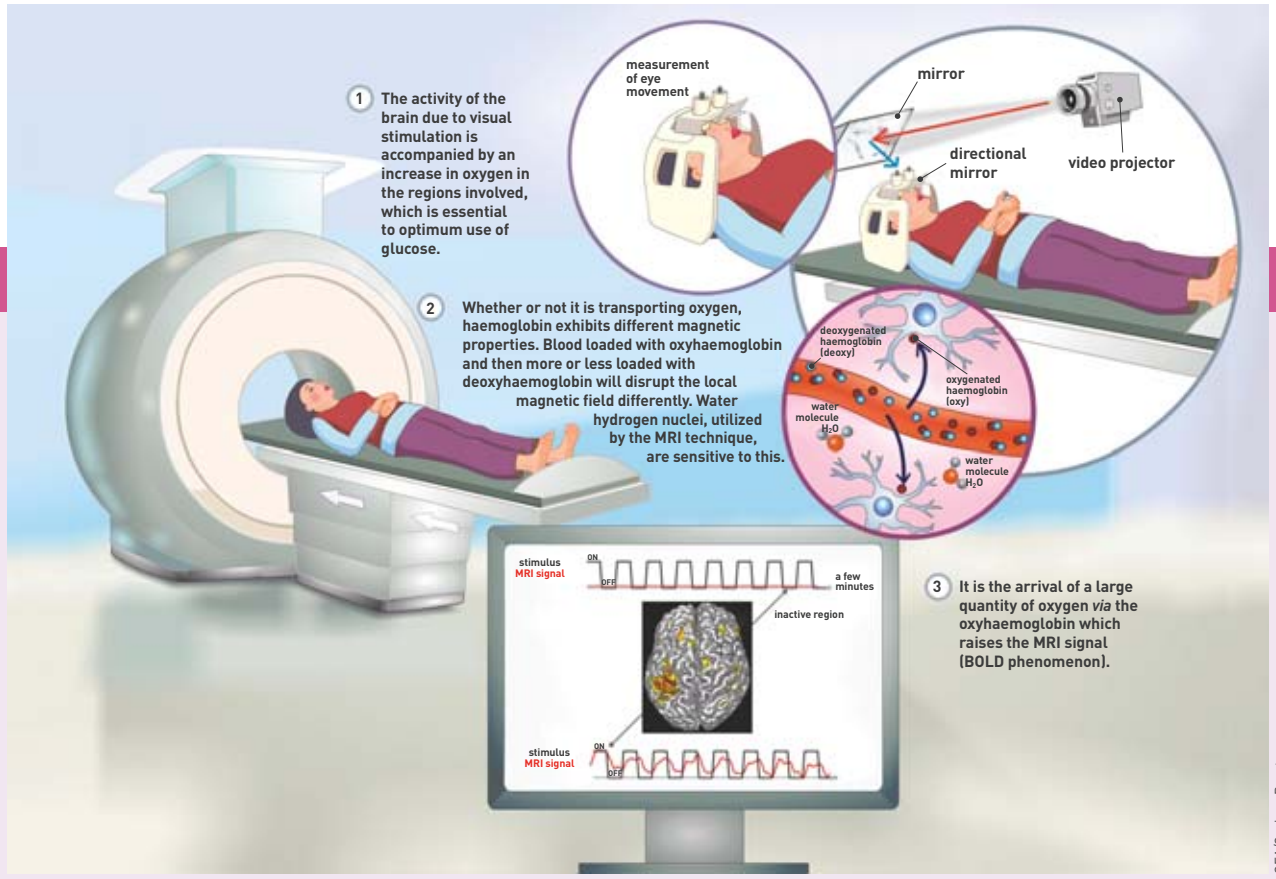
(1) Félix Bloch and Edward Mills Purcell received the Nobel Prize in physics in 1952.

(2) The tesla (T), a unit of magnetic field, represents about 20,000 times the value of the Earth's magnetic field in Paris.

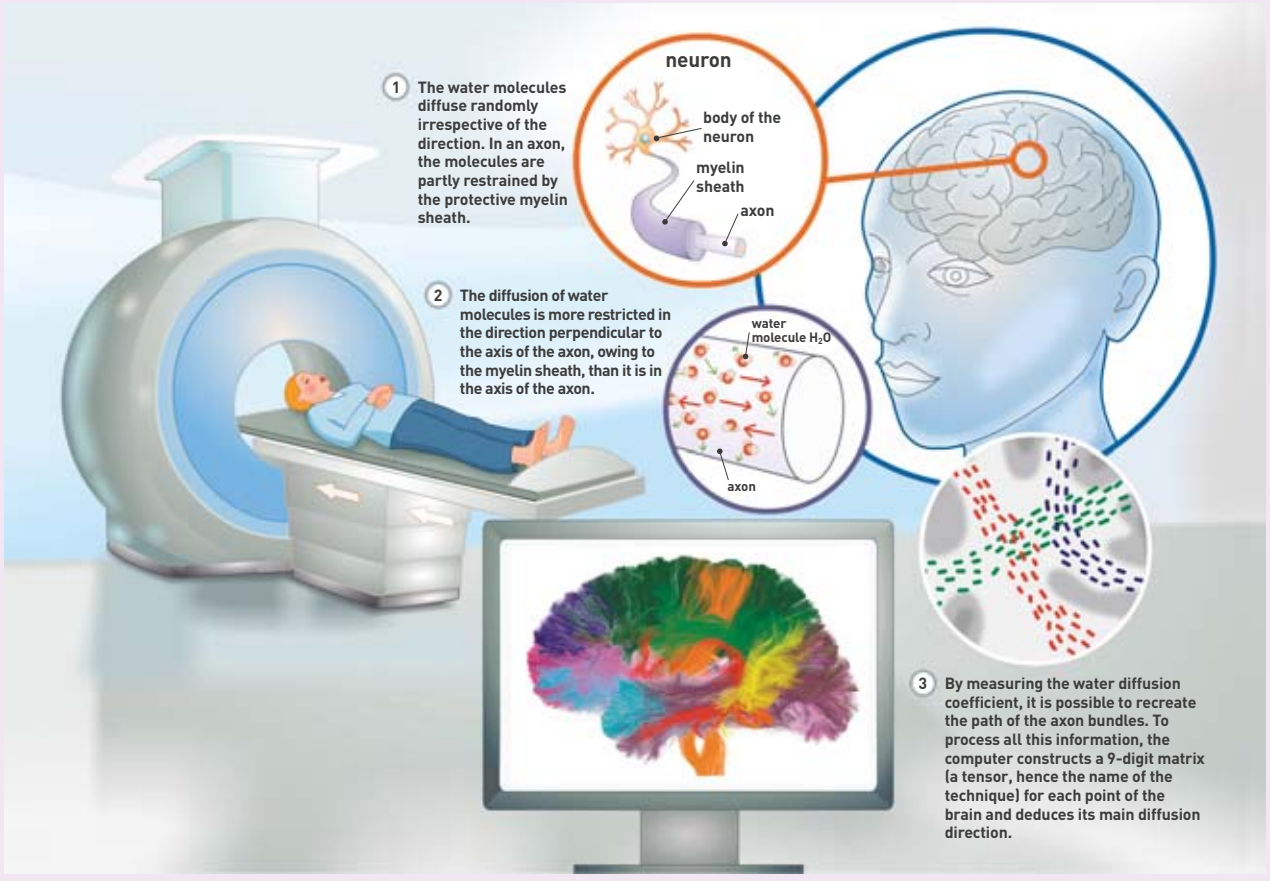


CEA/Corinne Beurtey

Principle of anatomical Magnetic Resonance Imaging (aMRI).



Principle of functional Magnetic Resonance Imaging (fMRI).



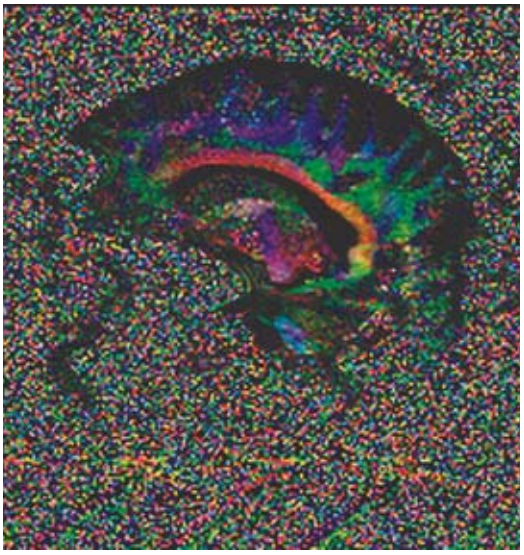
Principle of diffusion Magnetic Resonance Imaging (dMRI).

areas of the brain do not appear darker, as one might have expected, but lighter: the local increase in blood flow in fact more than compensates for the increased oxygen consumption. In any case, it was now possible, without injection, without radioactivity and without a cyclotron, to detect the activated areas of the brain (see box on *Magnetic resonance imaging*, p. 14).

There were however still a number of limitations. With a spatial resolution of a few millimetres and a time of a few seconds between the beginning of brain activity and the maximum signal, functional MRI easily out-performed PET… but did not achieve the level of detail of the functional neuronal units nor the rapidity of the cognitive phenomena. Moreover, the considerable noise of the device made it hard to carry out studies involving auditory tasks – therefore oral language or music based protocols in particular – and it was impossible to introduce any items liable to be magnetized in the field of the magnet. Apart from these few exceptions, BOLD MRI replaced PET for cognitive studies. The neurosurgeons also use it to identify the areas in their patients to be treated, or indeed to be avoided, during an operation.

The wiring of the brain

Despite the detailed anatomical images, despite the identification of the activated areas of the brain, the fibres of white matter connecting the various areas of the **cortex** could not be reached by the neuroscientists. They had to wait until the 1990s and the arrival of a new mode of MRI to be able to observe the "wiring" of the brain. Its origins lay back in the 1960s when physicists then discovered how to measure the diffusion of water in a medium using NMR. In 1985, Denis Le Bihan at CEA proposed a new MRI mode, suggesting that water does not diffuse in the same way in all types of biological tissues. In association with the echo-planar acquisition technique, diffusion MRI – as it is named – was soon to find clinical applications. In 1989, Michael E. Moseley, from the University of California (San Francisco, United States), showed that it could reveal a cerebral ischemia very early on, before the damage becomes irreversible. It then became the tool of choice for dealing with strokes. It was subsequently adapted for detecting cancerous tissues.



P.-F. Grosjean/CEA

Tractography image obtained on the 7-tesla MRI system at NeuroSpin. This diffusion acquisition sequence is able to visualize the orientation of the bundles of white matter fibres on a colour scale. Based on these orientations the fibre bundles themselves are reconstructed.

In 1990, Michael E. Moseley was back in the limelight: he noticed that within the white matter, water did not diffuse in the same way in all directions. This anisotropic behaviour is doubtless due to the fact that the water moves more easily along the **axons** than it does through the impermeable **myelin** sheath surrounding them. In 1992, the American biophysicist Peter J. Basser and Denis Le Bihan built on this result to lay the foundations of "diffusion tensor" MRI. It was only towards the late 1990s, thanks to new computerized techniques for reconstructing images, that it became possible to continuously plot the path of the nerve fibres. We then talk of **tractography** (see box on *Magnetic resonance imaging*, p. 14). In addition to its obvious benefits for neuroscience, this technique is also of interest to clinicians in dealing with white matter pathologies, such as multiple sclerosis. "Wiring" anomalies have also been identified in certain pathological conditions: schizophrenia, autism, Alzheimer's disease, chronic alcoholism, etc.

The successive technical advances in imaging – sometimes as a result of chance discoveries – have thus opened up a range of new fields for the neuroscientists. The brain is no longer a black box and it can today be observed in detail, its functioning examined and its organization understood. No matter how spectacular these advances, there is still room for progress. This in particular would mean achieving spatial and temporal resolutions corresponding to the scale of the functional cerebral phenomena.

> **Denis Le Bihan**[1], **Claude Comtat**[2], **Virginie van Wassenhove**[3] and **Isabelle Texier**[4]

[1]Institute of Biomedical Imaging (I2BM) – NeuroSpin
Life Sciences Division
CEA Saclay Centre

[2]Institute of Biomedical Imaging (I2BM)
CEA-Frédéric Joliot Hospital Service
Life Sciences Division
Orsay

[3]Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

[4]Leti Institute (Laboratory of Electronics and Information Technologies)
Technological Research Division
CEA Grenoble Centre

Looking to **the future**

How are we to push back the current limits of cerebral imaging? **The race for improved image definition, both spatial and temporal, is continuing. Although in appearance linear, this progress can in reality involve technological leaps and even changes in the fundamental scientific hypothesis.** Furthermore, using a single imaging mode does not always provide enough pertinent clinical or scientific data. Hence the temptation to combine several techniques in a single device, or a single installation...

P. Stroppa/CEA

Device combining Positron Emission Tomography (PET) and Computed Tomography (CT) installed on the Cyceron biomedical imaging platform in Caen (Calvados *département*). Feedback from a decade of operation of these systems, primarily in oncology, enables us to assess the added diagnostic value of integrated bimodality by comparison with examinations acquired using two separate machines. Hence the emergence of a new generation of multimode imaging.

Although nobody can really predict the future, a number of "weighty" evolutions in cerebral imaging are already taking shape for the coming years. For example, is it possible to improve the **spatial resolution** of MEG and EEG, two old and relatively "light-weight" techniques which are however incomparable in terms of **temporal resolution**? The most obvious solution would be to combine them, to create MEEG. Increasing the number of sensors placed on the scalp increases the number of signals and thus facilitates "inverse problem" resolution, in this case, the localization of the sources. In addition, magnetic information, which is unaffected by the presence of the skull and the scalp, enables the deformations of the electrical field to be corrected. The French **NeuroSpin** laboratory, in Saclay, thus uses an installation combining 204 gradiometers and 102 magnetometers for the MEG and up to 256 electrodes for the EEG.

Magnetism: new tools

The oscillatory nature of the electromagnetic activity of the brain has been known for a long time and characterizes general states of consciousness (wakefulness or sleep for example). More recently, there appeared the idea that groups of distinct **neurons** with a synchronous activity, in other words oscillating at the same frequency, could have a functional link – at least temporarily – and could for instance simultaneously process different aspects of the same object. New signal analysis software tools capable of identifying these synchronisms in the mass of data are currently being studied. The aim is therefore to reveal cerebral connections that are functional and perhaps even anatomical, even if this is beyond the reach of EEG.

Extremely sensitive and less costly magnetometers have also been developed for MEG and are currently

Very low field detectors

MagnetoEncephaloGraphy (MEG) offers totally passive, electrode-free detection of the magnetic field induced by **neuronal** activity (see box on *Magnetoencephalography*, p. 9). On the surface of the skull, this field has a very low intensity of a few tens to a few hundred femtoteslas – 1 fT = 10^{-15} T. The main type of magnetometers capable of measuring such signals, one billion times weaker than the Earth's magnetic field, are SQUIDs (Superconducting QUantum Interference Devices). They use the **quantum** properties of **superconducting** materials and must be cooled down to the temperature of liquid helium (4 K) in order to attain the required performance. Commercial systems comprise an array of about 300 sensors placed around the entire skull, inside a cryostat filled with liquid helium. In addition, owing to the weakness of the signals from the brain, the MEG system is installed in a shielded chamber significantly reducing environmental magnetic disturbances (power lines, moving vehicles, public transports, etc.).

A new type of highly sensitive magnetometer has recently been developed by the Condensed Matter Physics Laboratory (SPEC: *Service de physique de l'état condensé*) in the Physical Sciences Division (DSM: *Direction des sciences de la matière*) at CEA, in order to propose an alternative technique that only requires cooling to the temperature of liquid nitrogen (77 K). This solution is less costly, both in terms of consumption and manufacture, because the design of a tight liquid-nitrogen cryostat is far less complex than for helium. This "mixed" sensor relies on spin electronics, utilizing the electron's transport properties not only according to its electrical charge, but also its **magnetic moment** or **spin**. The transport properties can thus be "manipulated" in thin magnetic layers and by an external field. These principles also gave birth to "Giant MagnetoResistance (GMR)", in which the resistance of a set of thin films varies with the applied magnetic field[1]. For uses in biomagnetism, with very low fields, this magnetoresistive element is associated with a superconducting flux concentrator, which considerably increases

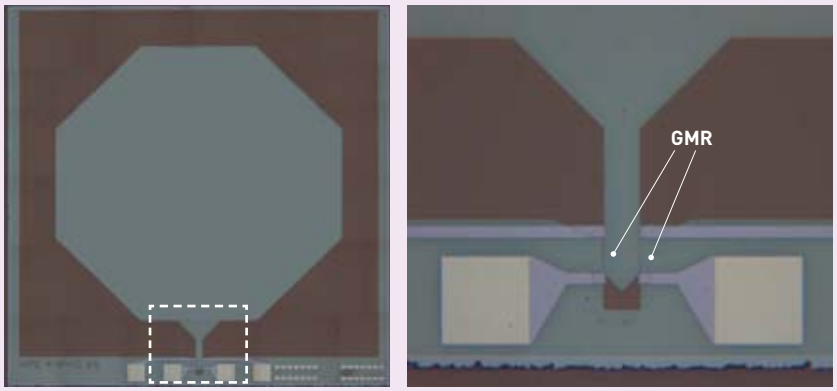


Figure 1.
On the left, an overview of a mixed sensor (measuring about 8 mm on a side). The dark brown part corresponds to the superconducting YBCO flux concentrator, operating at 77 K. The magnetoresistive elements (GMR) are positioned at the bottom and centre of the loop, with the light squares representing the GMR points of contact. On the right, a detailed view of the central part containing the two GMR elements.

being tested (see box on *Very low field detectors*, p. 18). A new type of probe, designed for highly localized detection of the magnetic signature of neuronal activity is also under development in the Condensed Matter Physics Laboratory (SPEC: *Service de physique de l'état condensé*) of the Physical Sciences Division (DSM: *Direction des sciences de la matière*) at CEA, in collaboration with the **INESC-MN** in Lisbon (Portugal). No study of this type has yet been carried out on the scale of the neuron, owing to the lack of adequate sensors. It is now possible, using the properties of **spin**electronics, to make sensitive and **micrometric** scale magnetic field sensors, capable of being integrated into systems resembling the electrodes utilized in electrophysiology. Under a partnership with **UNIC-CNRS** in Gif-sur-Yvette (Essonne *département*), the **Ernst Strüngmann Institute** of Frankfurt (Germany) and the **Aalto University** of Helsinki (Finland) for the modelling and validation of *in vitro* and *in vivo* tests, the European Magnetrodes (FP7-FET)[1] project, which started in early 2013, will offer a tool for observing neuronal activity on a small scale, thus ushering in the era of magnetophysiology.

(1) Address of the site: <www.magnetrodes.eu>. FP7 (Seventh Framework Program for Research and Technological Development)-FET (Future and Emerging Technologies).

(2) This concerns the brain, but also the pelvic cavity.

(3) In other devices, both tunnels remain distinct but aligned and the supine patient is transported from one to the other.

PET: combining for a clearer view

PET provides valuable functional data, particularly in **oncology**. However, given its low spatial resolution, most of today's clinical systems combine PET and CT, with the latter providing precise anatomical localization of the pathological centres detected by PET. The coupling of PET with CT is not always the most pertinent, in particular for cerebral imaging, because the CT images suffer from poor contrast in the soft tissues[2]. In this particular case, anatomical MRI is better than CT. In addition, contrary to CT, MRI is not restricted to the anatomy and can cover functional or molecular aspects. This is why a new generation of appliances is today emerging, integrating a positron emission tomograph with a magnetic resonance imager[3]. It then for example becomes possible to correlate the regional activations measured using functional MRI with the local biochemical variations of **neurotransmission** highlighted by PET. Multimode PET-MRI is still in its infancy and the first hybrid systems were only installed in 2011.

detection capacity. This latter can be made either from a material such as niobium (Nb) working at the temperature of liquid helium, or from an oxide of yttrium, barium and copper (YBaCuO, abbreviated YBCO), the superconducting properties of which occur at the temperature of liquid nitrogen. The magnetometers produced in this way have been evaluated and demonstrated detection performance of about ten femtoteslas (figure 1).

These mixed sensors were able to measure cardiac magnetic activity (MagnetoCardioGraphy or MCG) in healthy volunteers at NeuroSpin. Dynamic mapping was produced using recordings taken at different points above the torso, demonstrating the ability of these devices to capture biomagnetic signals (figure 2). The recordings were taken with some sensors cooled with liquid helium and others working with liquid nitrogen. The first MEG tests are currently ongoing. These sensors are also being used at present to detect NMR and MRI signals as part of a number of very low field experiments (10 mT).

> Myriam Pannetier-Lecœur
Saclay Institute of Matter and Radiation (Iramis)
Physical Sciences Division
CEA Saclay Centre

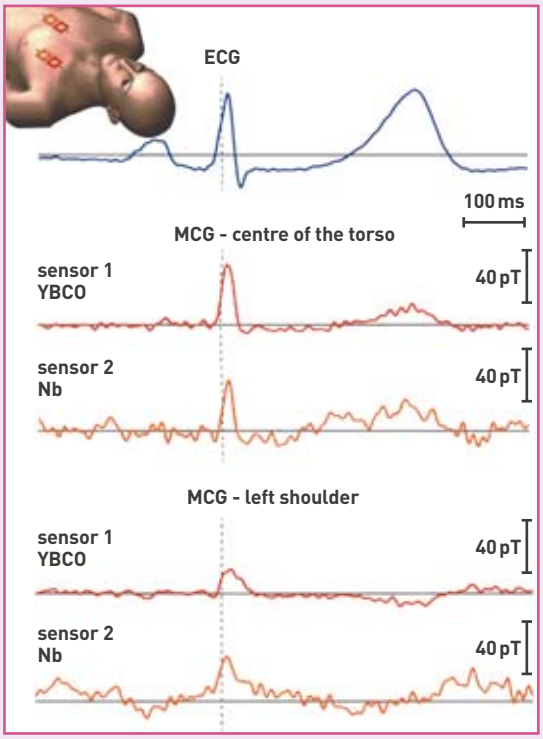


Figure 2.
MagnetoCardioGraphy (MCG) recordings taken at two locations above the torso of a healthy volunteer. The reference ElectroCardioGram (ECG) signal is shown in blue. The MCG signal is recorded in the centre of the torso and at the left shoulder using two sensors: one with a YBCO loop (in red) and the other with niobium Nb (in orange); 1 picotesla (pT) = 10^{-12} T. The exact positions of the sensors are shown on the volunteer's torso.

(1) This giant magnetoresistance is the basic principle used in computer hard disk read heads.

Siemens AG

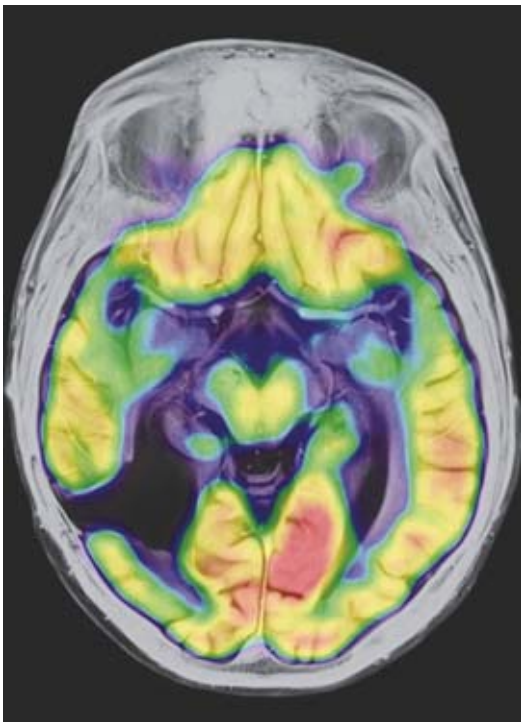
The Biograph mMR, a hybrid PET-MRI system developed by the Siemens Healthcare company. The Positron Emission Tomograph (PET) is integrated into a 3-tesla Magnetic Resonance Imager (MRI). This device offers simultaneous 3D acquisition of the whole body, thus opening up innovative avenues for the study of cerebral functions, of the physiopathology of cancers and so on. The Biograph mMR was the first hybrid PET-MRI system installed in France, at the CERMEP in Lyon, in the summer of 2014.

Instrumental challenges

Bimodal PET-CT, which is today commonly used, had no direct influence on the choice of the three main elements of a PET examination: the **radiopharmaceutical** injected into the patient, the **gamma photon** detector and the digital processing applied to the data. This is no longer the case when integrating PET into an MRI, which represents new instrumental challenges. Since the 1970s, PET detectors have consisted of a scintillating crystal(4) coupled with a photomultiplier tube. These tubes cannot however function in the presence of a magnetic field... It is therefore necessary to develop new detectors compatible with utilization in an MRI device. Various technological options are currently being evaluated. One of them, the Silicon PhotoMultiplier, or SiPM, is looking promising. The SiPM is immune to the magnetic field and far more compact than a photomultiplier tube. The first tests have shown that it is possible to install SiPMs coupled with scintillating crystals actually inside the MRI magnet tunnel, without significantly degrading the overall performance. The need for hybrid PET and MRI thus gave fresh impetus to gamma photon detection instrumentation research.

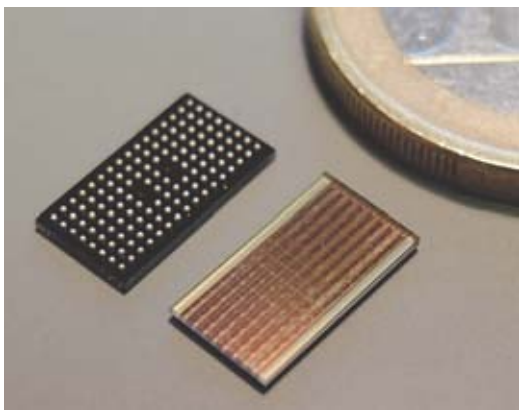
Unprecedented opportunities

These hybrid PET-MRI systems will probably offer new opportunities for diagnosis and biomedical research, in particular for understanding physiopathological processes and evaluating therapeutic mechanisms. However, in order to derive most benefit from these multimode systems, it is important to avoid simply juxtaposing information of different types, as is currently the case. A new field of research into biomedical data processing is therefore now opening up: the aim is to define and implement software capable of jointly acquiring and analysing these data which, although heterogeneous, are spatially and temporally correlated, in order to obtain additional information. The combination of PET and MRI will also influence the use and development of radiopharmaceuticals. Multimode uses can give a more precise estimate of the **metabolic** or biochemical parameters extracted from the PET images, for example through the association of a cerebral perfusion measurement obtained with MRI(5). Another avenue of research is the design of multimode PET-MRI probes: a single molecule labelled with a **positron** emitter also leading to increased contrast in MRI. A probe such as this would combine the complementary advantages of both modes: the sensitivity of PET and the high spatial resolution of MRI.



Siemens AG

Cerebral image acquired with the Biograph mMR hybrid PET-MRI system from Siemens Healthcare, for evaluating the efficacy of treatment of a tumour.



Sara Pellegrini/STMicroelectronics

Development of an SiPM sensor for Positron Emission Tomography (PET) compatible with Magnetic Resonance Imaging (MRI), as part of the European SPADnet project. This SPADnet1 sensor is manufactured with an STMicroelectronics 130 nm CMOS commercial image sensor process. The silicon chip measures 5.4 x 9.8 mm^2. On the left, the rear face with TSV (Through Silicon Via) solder balls and, on the right, the front face. The Leti Institute (Laboratory of Electronics and Information Technologies) at CEA was in charge of assembling and characterizing this sensor.

MRI: more precise imaging

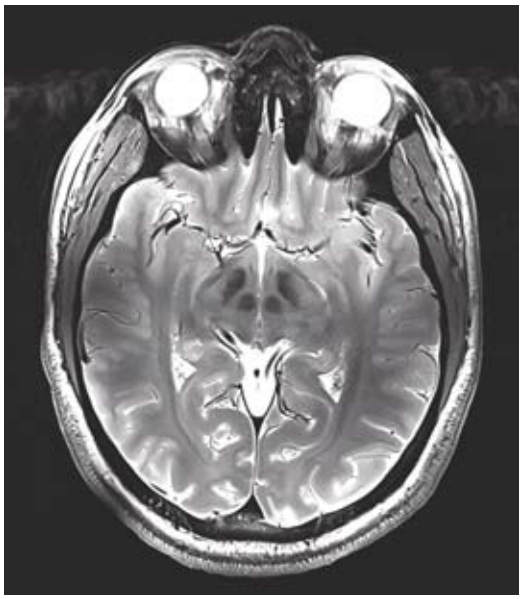
With regard to "pure" MRI, several avenues for progress have been developed. The most obvious one conceptually is the use of very high magnetic fields, an area in which development has been continuing since the 1990s, for two reasons. Firstly, because the intensity of the signal increases with the magnetic field, hence improved spatial and/or temporal resolution(6): the objective is to achieve a tenth of a millimetre or a hundredth of a second, and thus gain a factor 10 improvement over the best devices at present in service. In spatial terms, for example, the current resolution of about one millimetre still represents "packets" of several million neurons. Conversely, certain invasive methods such as

(4) Material which emits light when it absorbs photons or charged particles.

(5) Perfusion MRI is able to give a relative and/or absolute measurement of the cerebral microvascularisation parameters such as regional blood volume, average transit time and regional blood flow.

(6) Depending on what the device is being used for, preference will be given to one or other aspect, with the final overall result being a compromise.

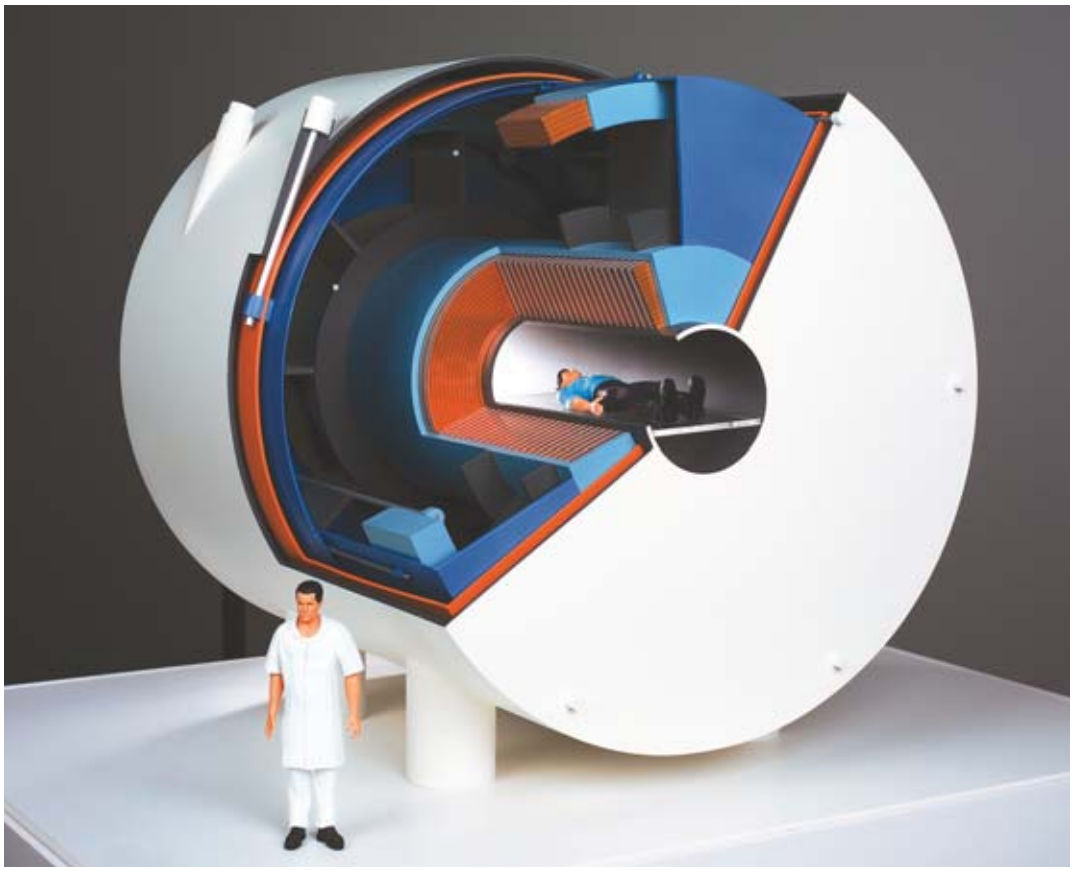
(7) The 17-T imager at NeuroSpin, designed for small animals, is also capable of this.



P.-F. Grosjean/CEA

Image acquired on NeuroSpin's 7-tesla MRI system. The details of the anatomy of the brain, such as the white matter, the cortex, etc., are shown with great clarity. The stronger the magnetic field, the more structural details are visible and the better the quality of the images. The use of very strong magnetic fields is one solution for pushing back the current limits of cerebral imaging. It will then be possible to observe the brain and its pathologies with even greater precision, on a scale more representative of the cellular and molecular phenomena that drive it.

micro-electrodes can show the activity of individual cells[7]. Yet it would appear that the functional units – the groups of coherent neurons functioning together at a precise moment for a specific task – are of intermediate size, approximately several thousand cells. Achieving this scale, which could well be the key to the working of the brain, is a major technological challenge, but one that is being taken up by CEA and its industrial partners as an 11.7-T device, the only one of its kind in the world, will be delivered to NeuroSpin in 2015. To give a little historical perspective, the first MRI device had a field of 0.1 T. The MRI scanners commonly used in clinical applications have a field of 1.5 T and, since the 1990s, several thousand of them exhibit 3 T. Around the world there are also about forty 7-T devices and... four of 9.4 T (two in the United States, two in Germany). The other benefit of increasing the magnetic field is that it would allow to "see" more than just hydrogen, and thus water. Elements such as carbon, oxygen, sodium or phosphorus would then be within the reach of these devices. For example, carbon naturally comprises about 1% of a particular **isotope**, ^{13}C, which gives a magnetic resonance signal that is currently undetectable because it is too small. With intense field devices, a whole world of biological molecules, in particular the neurotransmitters, would become observable



P.-F. Grosjean/CEA

Longitudinal section of the 11.7-tesla magnet and the cryostat assembly, developed under a Franco-German partnership, in which CEA's Institute of Research into the Fundamental Laws of the Universe is a stakeholder. It is intended for the Magnetic Resonance Imaging (MRI) system which is to be installed at NeuroSpin in 2015. This intense field MRI system, dedicated to whole-body studies of humans, will push back the boundaries of knowledge even further. With an increasingly strong magnetic field, it will become possible to study the working of the brain on a scale of a few thousand rather than a few million neurons.

without having to synthesize and inject analogues labelled with radioactive elements, as is currently the case with PET.

Watching neurons work

The other major area of cerebral MRI development is based not on a technological leap, but on the use of a hitherto neglected biophysical phenomenon. Functional exploration today essentially relies on neurovascular coupling (the fact that blood flow increases in the activated areas of the brain). For several decades now, it has also been observed that the neurons themselves – and maybe also the glial cells – swell when they are activated. This type of swelling necessarily slows down the diffusion of water, hence the hypothesis, formulated by Denis Le Bihan (CEA), that it would be possible to derive diffusion MRI images from this. Proof was provided in 2005, with the same type of experiment as that which demonstrated the feasibility of BOLD (Blood-Oxygen-Level Dependent) MRI: viewing a checkerboard. This new MRI mode has a number of advantages. First of all, the signal begins with the stimulus – rather than several seconds later as with BOLD – and stops immediately once the stimulus ceases. Moreover, the images are more detailed and better defined, because what is "seen" is not a change in blood flow – and thus a phenomenon affecting the vessels irrigating the activated region – but the neurons themselves.

In addition to these clearly defined tendencies, one cannot rule out that an as yet unknown technology will arrive and revolutionize cerebral exploration. After all, the history of medical imaging abounds in surprises and unexpected developments...

> **Claude Comtat**[1], **Myriam Pannetier-Lecœur**[2], **Virginie van Wassenhove**[3] and **Denis Le Bihan**[4]
[1]Institute of Biomedical Imaging (I2BM)
CEA-Frédéric Joliot Hospital Service
Life Sciences Division
Orsay

[2]Saclay Institute of Matter and Radiation (Iramis)
Physical Sciences Division
CEA Saclay Centre

[3]Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

[4]Institute of Biomedical Imaging (I2BM) – NeuroSpin
Life Sciences Division
CEA Saclay Centre

The FLI imaging research infrastructure

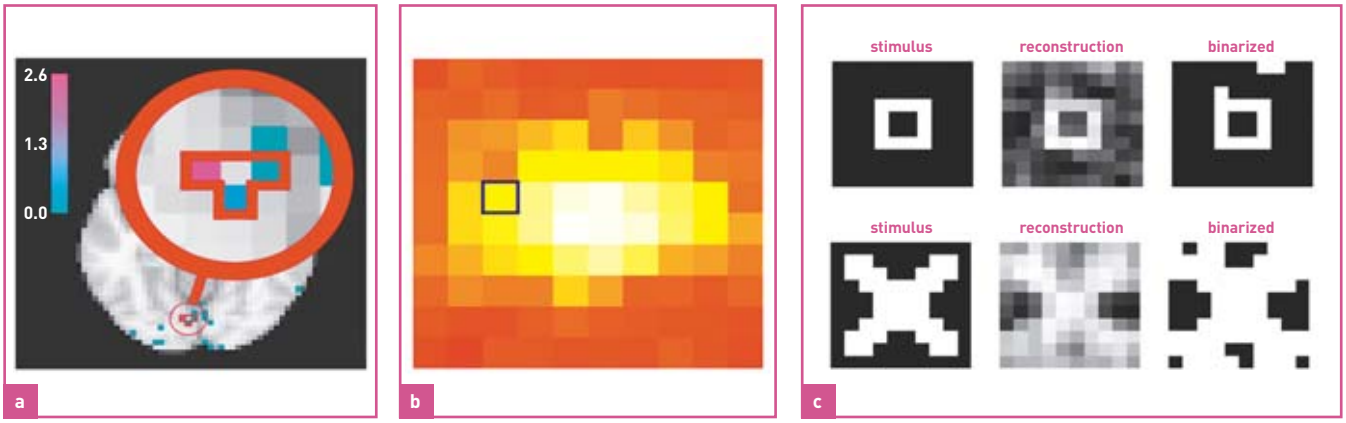
FLI – France Life Imaging – is an infrastructure created in 2012 by the Investing in the Future Program (PIA: Programme d'investissements d'avenir) to coordinate and harmonize French research and technology in biomedical imaging. The organization of this network of platforms is designed to allow access to cutting-edge imaging systems for all academic or industrial partners submitting a request *via* the FLI gateway or the regional nodes. All *in vivo* imaging modes are covered (Positron Emission Tomography PET, Single Photon Emission Computed Tomography SPECT, Magnetic Resonance Imaging MRI, optical imaging, ultrasounds, X-ray computed tomography). FLI comprises 6 regional nodes (Paris South, Paris Centre, Lyon, Grenoble, Marseille and Bordeaux) and a cross-cutting thematic node concerning the management and analysis of images and associated data. This latter meets the needs of the major population studies which are today essential when integrating the complexity and variability of the clinical pictures linked to numerous pathologies. FLI also proposes training for imaging stakeholders. Its structure is designed to promote and coordinate thematic research into imaging agents, biomedical instrumentation, image and data analysis, interventional imaging, and encourage the emergence of laboratory networks. FLI is thus extensively open to the community of imaging methodology researchers and those working in fields applied to biology, health and neuroscience, in order to overcome the current technological challenges to *in vivo* imaging.

One goal of FLI is to contribute to the construction of the European imaging research infrastructure and become the sole French partner for *in vivo* imaging in the European Euro-BioImaging network. FLI is thus able to offer a wide range of high-quality services for clinical and scientific researchers using imaging.

> **Régine Trébossen**[1] and **Franck Lethimonnier**[2]
Institute of Biomedical Imaging (I2BM)
Life Sciences Division
[1]CEA-Frédéric Joliot Hospital Service
Orsay
[2]Aviesan, Director of the Multi-Organization Thematic Institute (ITMO) Health Technologies

Cerebral decoding: the example of **vision**

New techniques for analysing the brain's activation signals take into account the activation profile of a set of regions of the brain simultaneously, rather than distinct areas. These techniques are collectively known as multivariate decoding and offer a level of sensitivity that is superior to conventional methods, to the extent that in certain cases, it is possible to infer the stimulus presented to the subject from the cerebral activation images...



CEA/NeuroSpin

Example of encoding/decoding analysis. These experiments to reconstruct stimuli from activity in the early visual cortex are carried out using the http://nilearn.github.io/ code and data from Y. Miyawaki *et al.* ("Visual Image Reconstruction from Human Brain Activity using a Combination of Multiscale Local Image Decoders", *Neuron* 60(5) (2008) 915-929). In a, a few voxels of the brain can predict the contrast of one pixel in an image, located in figure b. The colours of this figure indicate the quality of prediction which increases from red to white: it would appear that the central part is better predicted than the periphery. In c, images reconstructed from fMRI activation patterns: on the left, the stimulus used; in the centre, a probabilistic reconstruction which, once thresholded, gives an attempt at binarized reconstruction (right).

Functional imaging studies use experimental paradigms taken from **cognitive** neuropsychology to understand how the brain performs basic or complex functions. Cerebral images, reflecting the physiological state of groups of **neurons**, are acquired while the subject reacts to a clearly defined stimulus. For methodological reasons (background noise, spatial distribution of the information, etc.), several sets of images – obtained successively in the same subject or from different persons subjected to the same stimulus – must be analysed in order to improve the signal-to-noise ratio. Image processing involves mathematics, in particular statistics, in order to localize the regions in which the signal level is modified by the stimulus, and extract the cerebral network(s) involved. In conventional approaches, each region of the image is analysed independently of the others: the aim is to find out whether or not it is activated by the stimulus.

Improved sensitivity

Over the past decade, new image analysis techniques have been developed (often used with functional Magnetic Resonance Images, fMRI). These methods are collectively known as *multivariate decoding*(1) and consider the activation signal as a pattern covering a set of regions (of up to several voxels(2) or even the whole brain). The question therefore is no longer to analyse areas of the brain independently of each other, but to identify a general activation profile. Thanks to their increased sensitivity, they take into account information that conventional methods would have ruled out as irrelevant. This is particularly true because it is now possible to monitor a test on an individual brain and thus discern details which would otherwise have been "erased" by the necessary smoothing of data concerning several individuals or tests. The result is that these methods discriminate between extremely precise experimental effects, lifting the veil on the spatial organization of the neuronal coding of certain cognitive parameters (number, speed, colour, etc.), hitherto only accessible

(1) Multivariate decoding originated in mathematics, in shape recognition and in automatic learning. These are what are known as classification techniques, algorithms which detect regularities in data, thus enabling them to be classified. They are for example used for automatic reading of bank cheques or postal addresses. In this case, the algorithms are "trained" with preliminary data incorporating various handwriting styles for digits or letters.

(2) The voxel is a volumetric pixel which is the basic unit of an MRI image.

to invasive *in vivo* electrophysiology techniques[3]. They can also be used for temporal monitoring of the brain during the course of the experiment and, above all, the detail of the analysis is such that even if one cannot "read the thoughts" then at least – in certain experimental configurations – deducing the stimulus presented to the subject could be envisaged using fMRI data.

The case of vision

These powerful techniques were applied to the study of vision at a very early stage. It is thus possible to decode the orientation, direction of motion or colour of an object shown to a person in MRI, based on the activity of their early visual cortex. These results have triggered enormous interest because they show the presence of dimension-related information for which neurophysiology has highlighted a columnar organization on a fine scale (such as orientation, direction of motion, etc.) in the fMRI signal. For example, the neurons in the early visual cortex exhibit a preferential orientation in sub-millimetre columns. Although this scale is less than that of the spatial resolution of fMRI, the multivariate decoding methods can, from the activation profile, deduce what was the orientation of a stimulus presented to the subject. They also yield neuroscientific insights that would have been difficult to obtain by a mere mapping approach concerning the functional role of early visual areas in some cognitive processes. It has thus been proven that the early visual cortex takes part in maintaining information in the working memory[4], even if the overall cortical activity level is at the baseline. Another study has revealed that while anticipation reduces the intensity of the overall response in the early visual cortex, it improves and sharpens the representation of the stimuli in this area.

Representations and invariance

With regard to high-level vision[5], multivariate decoding can, in the occipito-temporal cortex, discriminate between activations linked to different objects belonging to the same category, even within extremely homogeneous categories such as faces or words. Another interesting aspect of this approach is that it enables to infer characteristics of the representation of a stimulus achieved at a given level of the cortical hierarchy, such as the question of invariance with respect to viewing conditions (figure 1). For example, a classification algorithm or classifier, initially trained to discriminate between

(3) Electrophysiology techniques are based on the implantation of very fine electrodes in the brain and recording the electrical activity of the neurons or groups of neurons within a region of a few tens of micrometres.

(4) Working memory: short-term memory capable of storing and manipulating new information for a period of several seconds.

(5) High-level vision in particular concerns shapes and categories of objects, while low-level vision deals with orientations and contours.

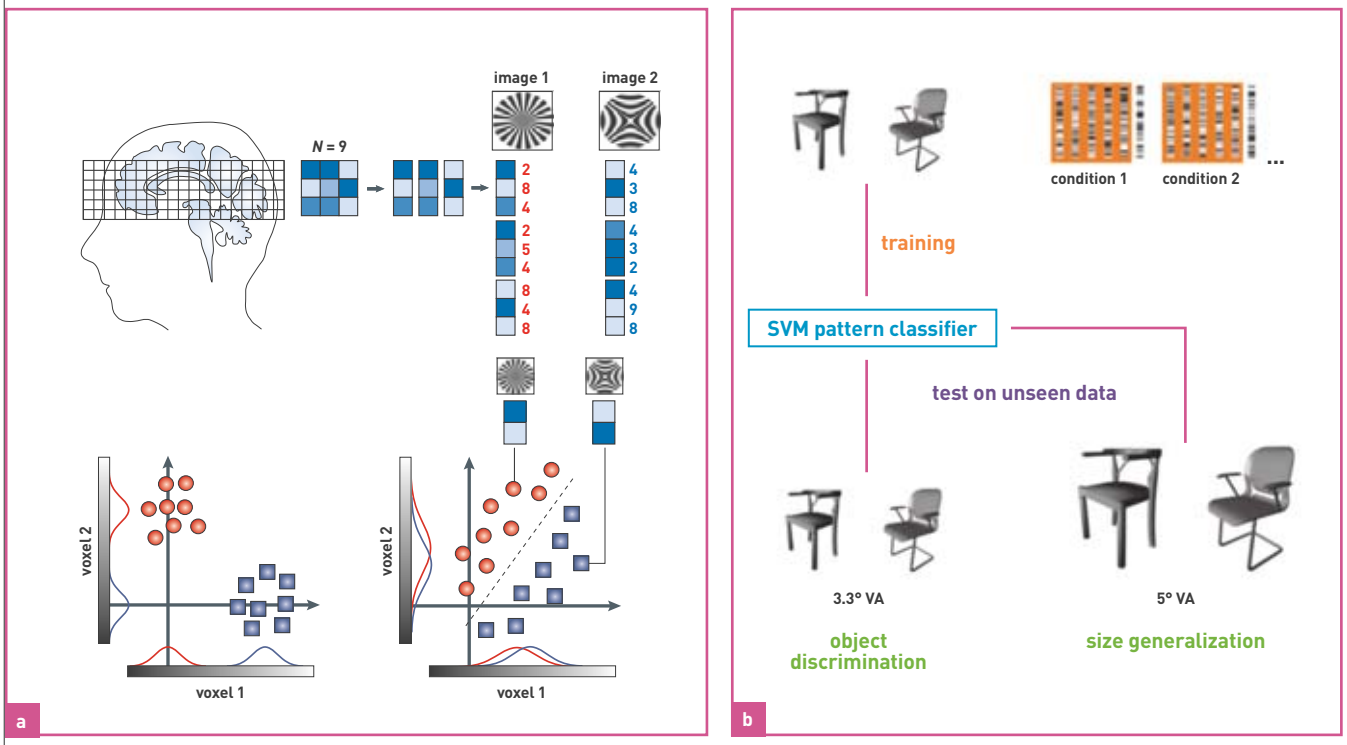


Figure 1.
In a, schematic illustration of multivariate classification in a two-dimensional space. At the top, fMRI measurements of brain activation in a subject looking at images, in a large number of basic volume units, or voxels. Activation in a sub-set of voxels, here 9, constitutes a profile, which is expressed in the form of a pattern vector reflecting the mental state of the subject. Each pattern vector can be considered to be a point in an *N*-dimensional space, where *N* corresponds to the number of voxels. In the diagrams at the bottom, the pattern is limited to two voxels (instead of 9) and the red and blue dots relate to the two conditions. At the bottom-left, the two voxels clearly indicate a preference for one of the two experimental conditions. A conventional voxel-by-voxel approach would thus be sufficient. At the bottom-right, the activations of the two voxels overlap and show no significant difference between the two conditions. The two stimuli can however be differentiated by taking into account the combination of responses. In b, principle of training and testing a classifier on data from different conditions to understand the characteristics of cerebral representation, in this case, the degree of size invariance for objects.

(a) adapted from J.-D. Haynes *et al.*, "Decoding mental states from brain activity in humans", *Nature Reviews. Neuroscience*, **7(7)** (2006) 523-534
(b) adapted from E. Eger *et al.*, "FMRI activity patterns in human LOC carry information about object exemplars within category", *Journal of Cognitive Neuroscience*, **20(2)** (2008) 356-370

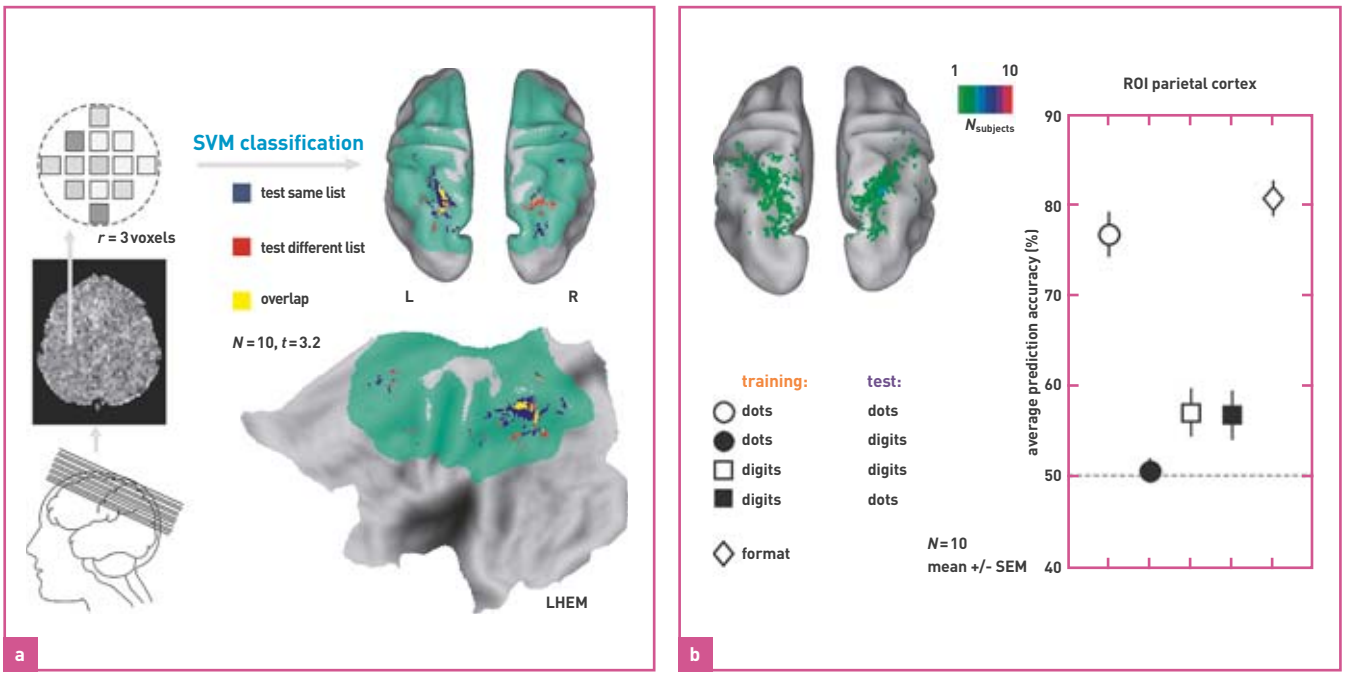


Figure 2.
In a, a local multivariate analysis (with a radius of 3 voxels) revealed that in a group of subjects (N = 10), the discriminating signal patterns of a given non-symbolic number (cluster of dots) were detected in the intra-parietal sulcus. The significance of the decoding accuracy maps was determined in subjects by a test t (the corresponding map here is thresholded at t = 3.2). The scale of the imaged volume is shown in green. The regions marked in blue and red correspond to places where discrimination between numbers is significant. "Test same list" and "test different list" refer to the different lists of stimuli used which equalled either the size of the dots, or the total number of pixels between numbers (this manipulation was used to ensure that the number discrimination in this experiment could not be explained by a trivial low-level effect such as brightness or size). In b, discrimination between numbers with non-symbolic (cluster of dots) and symbolic (digits) format in a Region Of Interest (ROI) of the parietal cortex [N = 10, mean +/- standard error (SEM)]. For each subject, the 1,000 voxels with the most activity for all the combined stimuli were chosen. The map on the left gives an idea of the regions included and their overlap in the subjects (the colour indicates the number of subjects for which a voxel is selected). On the right, the classification rate (chance = 50%) shows that the digits are less well differentiated than the numbers of dots. Nonetheless, a classifier trained in discriminating digits predicts the corresponding numbers of dots just as well, which indicates a cerebral representation of numbers which is at least partially format invariant.

adapted from E. Eger *et al.*, "Deciphering Cortical Number Coding from Human Brain Activity Patterns", *Current Biology*, **19(19)** (2009) 1608-1615

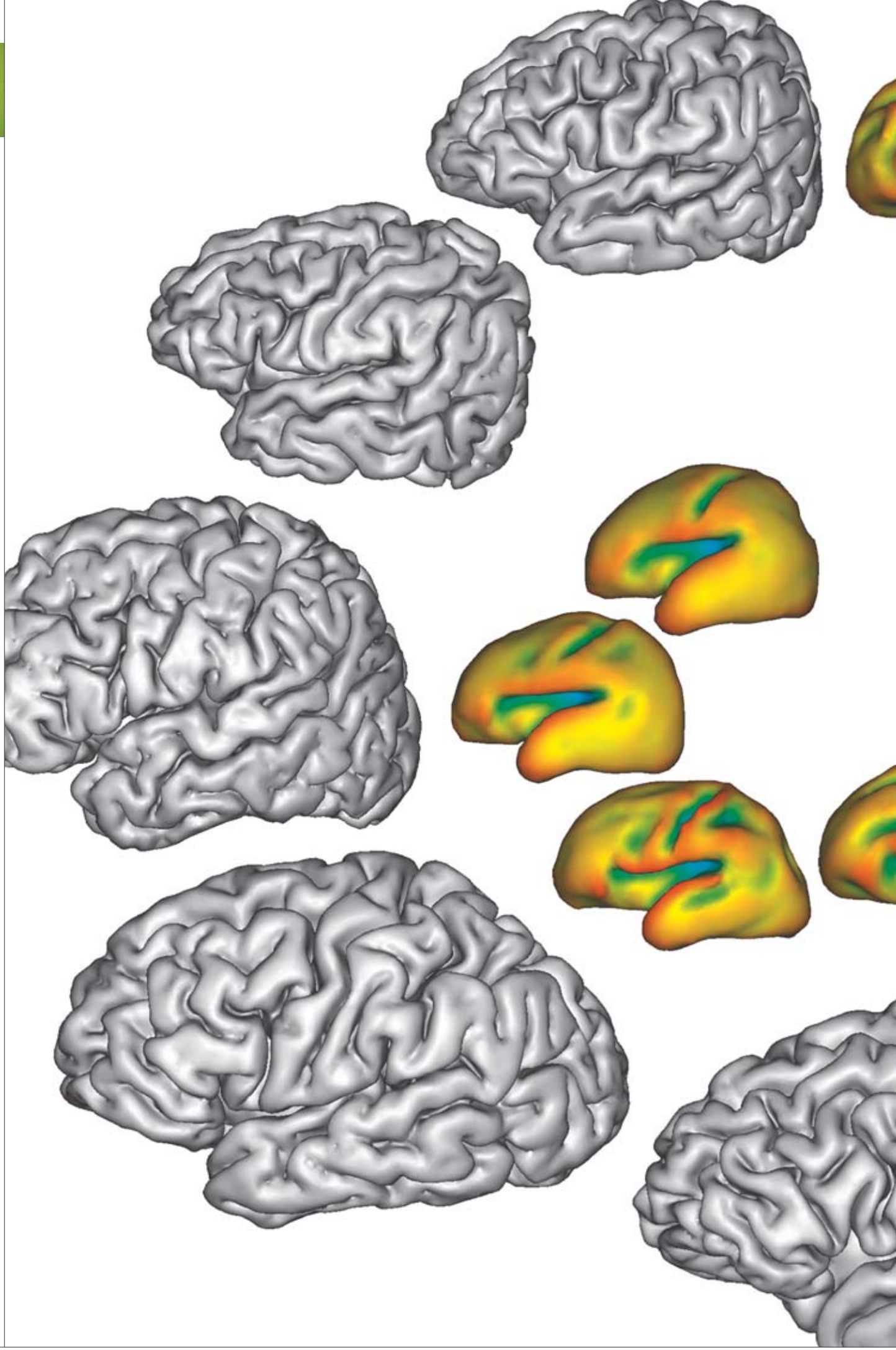
two objects, can be applied to functional imaging data acquired with a stimulus representing one of these objects in a new way, either size or viewpoint. If the object can be correctly classified after this change, this suggests an invariance in the cerebral representation with respect to this parameter (size or viewpoint) in the region of the brain concerned. An application to the representation of numbers is a good illustration of this approach. In this case, a classification algorithm was used to decode, in the dorsal visual pathway and the intra-parietal cortex, the number that a participant was seeing and holding in mind. Although trained to discriminate between numbers presented in Arabic digits, this classifier also successfully predicted the corresponding non-symbolic numbers such as a set of dots (figure 2). These discoveries are in line with the neurophysiological discoveries on the number selectivity of neurons in the parietal areas and show that in the human cortex, numbers are represented in a way that is partially format-invariant. This representation probably reflects the association of visual symbols with their meaning through learning.

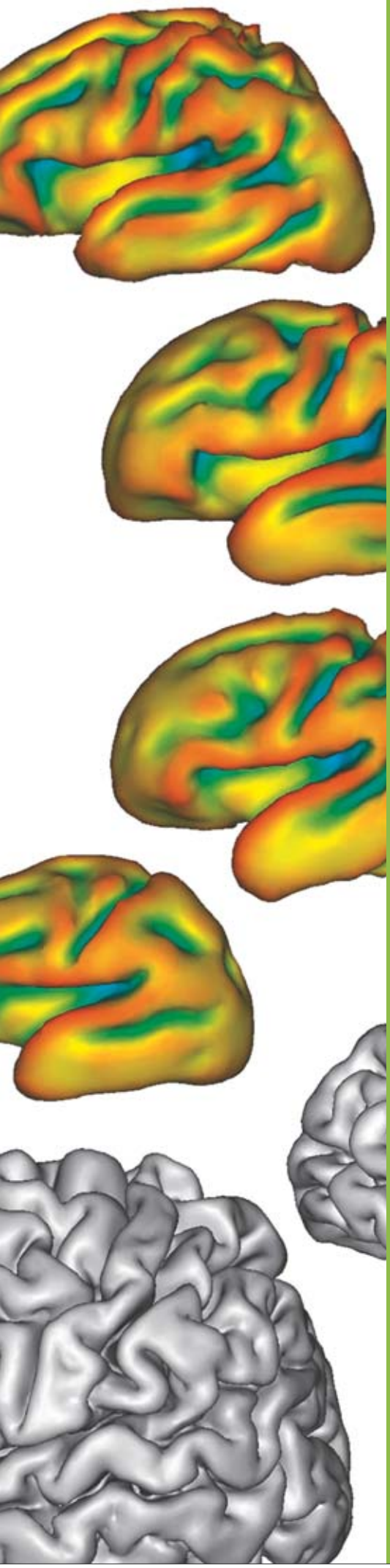
Strong potential

In the studies presented so far, multivariate decoding is used to classify cerebral activity according to pre-specified categories. Other work is attempting a complete reconstruction of the stimulus on the basis of the subject's brain activity, possibly using an *a priori* knowledge of all the possible stimuli (see illustration, p. 23). In short, the multivariate decoding techniques establish a link between the neurophysiological recordings on the one hand and conventional mapping-based neuroimaging on the other. They make it possible to infer what information is represented from the regional distribution and the local intensity of brain activation, recorded by means of functional imaging. The potential of these approaches will probably increase with the arrival of high **resolution** and/or high field imaging, while the development and improvement of efficient decoding algorithms is an active ongoing research area.

> **Evelyn Eger**[1] and **Bertrand Thirion**[2,3]
Institute of Biomedical Imaging (I2BM) – NeuroSpin
[1]Inserm U992 – Cognitive Neuroimaging Unit
[2]Information Analysis and Processing Unit
Life Sciences Division
CEA Saclay Centre

[3]Inria Saclay Île-de-France
Palaiseau





The stages of the growth of the human brain, from the premature newborn to the adult. In the premature newborn (in colour) the brain rapidly increases in size and number of folds. After 2 years, brain growth is far slower.

CEA – Inserm/J. Dubois and J.-F. Mangin

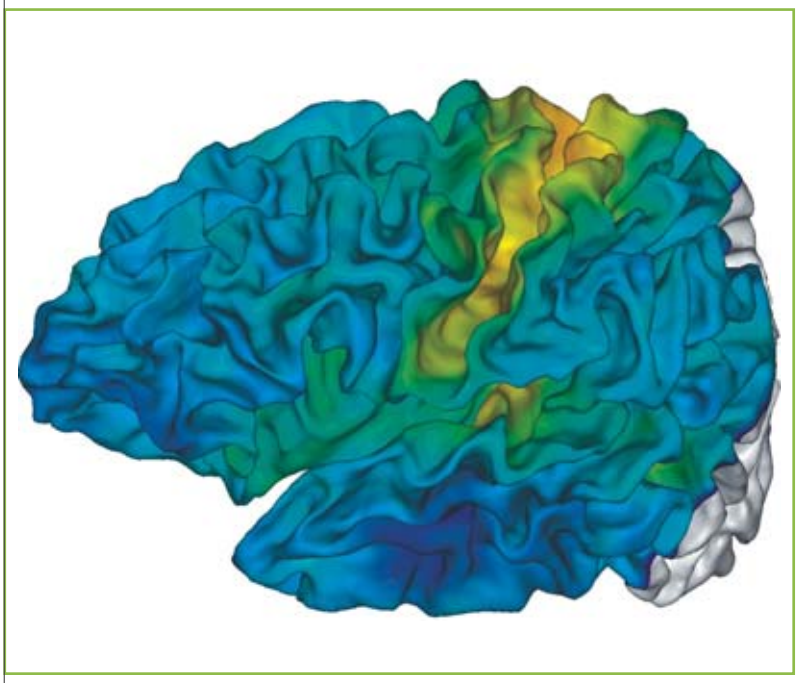
II. DEVELOPMENT, LEARNING AND PLASTICITY OF THE BRAIN

For a very long time, the brain of the child remained a closed box. It was only after the French doctor Paul Broca made the discovery in 1861 that his patient, Mr. Leborgne, had lost the power of speech because of a lesion in the left frontal region, that knowledge about the adult brain progressed rapidly, with the symptoms presented being linked to the cerebral lesions revealed by an autopsy. Fortunately, children are usually in good health. It was then necessary to wait for the development of non-invasive cerebral imaging in order to be able to study how the child's brain learned to speak, read or calculate. Electroencephalography and magnetoencephalography today enable us to follow, millisecond by millisecond, how information passes through the brain. Magnetic resonance imaging enables us to see the regions activated by a task and plot the fascicles linking the various regions of the brain. These techniques can be used in the very youngest subjects, even the foetus or premature newborns.
Child research is growing and revolutionizing our understanding of the brain. Through these studies the child no longer appears to just passively experience its environment, but is an active participant in learning. It does not simply absorb its environment, but anticipates it, right from the moment of birth. We are gaining a clearer understanding of how culture takes advantage of the brain's constraints and how the human brain acquires new capabilities, such as reading or music, by drawing on our natural capacity. These studies also shed light on the subtle mechanisms liable to be altered by developmental pathologies such as dyslexia or dyspraxia, and how the extremely heterogeneous and prolonged maturing of the human brain can explain the particularities of cognition in infants, children and adolescents. Finally, understanding how this highly complex organ develops gives us vital clues to learning about brain function in adults. These are the challenges to be met in the coming years with the help of cerebral imaging.

> Ghislaine Dehaene-Lambertz
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

How does the **brain develop?**

The setting of neurons and their connections, the emergence of a functional architecture with specialized networks, the learning, the emergence of complex cognitive capacity: the long development of the human brain has not yet revealed all its secrets. New imaging techniques usable on children of all ages, including premature newborns, are revealing the complexity of cerebral development, while at the same time challenging a number of previously accepted truths. These images confirm the latest hypotheses on the functioning of the brain: our "thinking machine" is above all a "prediction machine".



CEA – Inserm

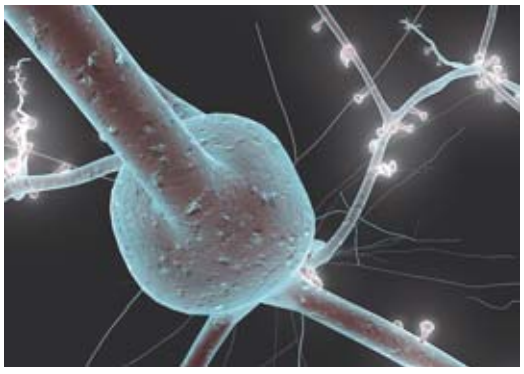
Image of the maturing of grey matter in an infant a few weeks after birth, coded using a colour scale. The yellow regions, corresponding to the primary motor and sensorial regions, are the most mature, while the darkest blue regions are the most immature. This image clearly shows the difference in maturing rate between the different cerebral regions.

Gradual setting

During the first months of life, the brain develops very rapidly. One need simply observe the development of the skull's circumference in the child's health records: it increases by 14 cm during the first 2 years of life and then by only 7 cm for the next 16 years. During the last months of pregnancy, the neurons gradually appear around the periphery of the brain, to form the **cortex** (or grey matter), and the contacts between them multiply. Inside the cortex, the neighbouring neurons become connected by their **dendrites**. Furthermore, the long **axon** of each of the cells provides a long-distance connection. These axons are grouped into fascicles joining different regions of the brain and constitute the **white matter**.

This entire structure is in place at birth (full term) and subsequent brain growth is mainly the result of thickening of the axons in the white matter, which are gradually covered by a sheath of **myelin** which accelerates nerve transmission. In the grey matter, it is the dendritic arborisation which becomes increasingly exuberant and gradually increases the distance between the neurons. This multiplication of contacts, or synaptogenesis, is one of the ways in which the brain learns. The **synapses** multiply randomly, but those which are least used disappear.

How is a "learning machine" manufactured? How are cells arranged and connected to create thought? What type of calculation must they perform to understand a poem by Rimbaud, a differential equation or build a spaceship to journey to Mars? All of this is done by the 100 billion or so **neurons** arranged in six layers in the **grey matter**, around the periphery of the brain. An assembly of fibres interconnects neighbouring neurons, but also neurons that are a few centimetres apart. A subtle combination of chemical and electrical signals, whether between neurons or with the **glial cells**(1), controls the complex setting of this structure.



EPFL/Blue Brain Project

Neuron in 3D. At birth, a baby possesses about one hundred billion neurons which will no longer multiply, except in a few very particular regions. Its brain has not however developed fully, because the synapses have barely begun to form. There are different types of neurons, each with particular inhibition and excitation properties. The fine-tuning of the communication between these neurons takes place throughout a person's lifespan, but especially during childhood.

(1) Scientists are today beginning to discover the importance of these cells in the working of the brain, after they had for a long time been considered as having a purely support function.

This proliferation/elimination gradually leads to a fine-tuning of the connections and stabilization of the information pathway. This process, which continues throughout a person's lifespan, is particularly intense for the first 20 years, the period during which we take advantage of this fact to send our children to school!

A functional architecture that is complex from the outset

Two other concepts are essential in understanding cerebral development. Firstly and contrary to common opinion, the brain is not a formless structure waiting to be modelled by its environment. However, it has often been compared to a computer. Both are indeed "calculators" but, unlike what happens in a computer, the regions of the brain do not all do the same thing. The structure of the connections and the direction of the flow of information force the type of calculations performed into a given location in the brain.

From the moment it becomes possible to watch it function, that is around the 6th month of pregnancy, at a time when the neurons are still migrating to their final locations, the brain reveals a complex functional organization. The responses to syllables are for example astonishingly similar in adults, children and a premature infant born three months before term. One way of visualizing the functional organization of the brain lies in the fact that the neurons are never at rest and that the activity of the connected neurons varies synchronously. In imaging, this synchronous activity is reflected by slow variations in the MRI signal, which is sensitive to the **deoxyhaemoglobin** levels in the vessels irrigating the cortex. In adults, a number of networks involving large regions of the brain have thus been isolated (visual, auditory, sensorimotor, executive, etc.). These networks can be found in premature newborns and are very similar to those of adults. It is not therefore the outside world that organizes the brain but its own particular organization that enables it to efficiently take advantage of its environment.

Varying rates of maturity

The second major characteristic of the human brain is that its development is not only spread over a long period of time, the first fifteen years of life, but is also extremely heterogeneous. The **primary regions** mature rapidly. The primary visual regions thus reach the adult state at the end of the first three months of life, while the frontal and parietal regions continue to develop until the end of puberty. These latter are involved in planning of actions, executive control, reflection and explicit learning. Owing to this significant difference in maturity times, scientists believed for a long time that these regions, involved in abstract **cognitive** operations, were little used by the infant, if at all. Cerebral imaging has shown the contrary. These regions take part in the baby's thoughts very early on, but are extremely slow. For example, studies conducted at **NeuroSpin** have shown that awareness responses to a stimulus from the outside world, observed at about 300 milliseconds (ms) in adults, are three times slower at 12 months (900 ms) and even slower at 5 months. Thanks to the acceleration of these networks during the cerebral maturing process, the infant will increasingly be able to control what is happening around it.

CEA – Inserm

Visualization of the large fascicles of white matter connecting the functional regions of the brain in an infant, using diffusion MRI. About twenty years will be needed for cerebral connectivity to become fully mature.

A prediction machine

Observation of an activity in the frontal regions in infants and even newborn babies, has called into question the concept of "bottom-up" learning, in which the highest level(2) regions only begin to learn when the more primary regions have become efficient. On the contrary, it confirms current hypotheses on the functioning of the brain. Instead of seeing the brain as reacting to external stimuli, these hypotheses consider it to be a prediction instrument. Our brain would seem to be permanently calculating which should happen according to what has happened so far, and learning would be the result of prediction errors. These prediction systems would appear to be present at all levels of the cerebral hierarchy, from the most primary regions up to the most abstract. These concepts are revolutionizing our view of cerebral development, by postulating that the high-level regions could learn before the low-level regions and even guide them and help them to learn. Our research in infants exactly tallies with these theories.

We are still a long way from completely understanding how the human brain develops, but the host of new imaging techniques that can be used on children with no risk, no matter how young, finally gives us the opportunity to study the setting of this wonderful learning machine. Maybe one day we will understand how human thought is constructed.

> **Ghislaine Dehaene-Lambertz**
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

(2) The *high-level regions* are associative regions and the *low-level regions* those which are not, such as the sensorial and motor regions.

The contribution of **neuroscience** to **learning:** the example of **dyslexia**

A significant proportion of children encounter learning difficulties which can compromise their professional lives. The most common – and most extensively studied – of these difficulties is dyslexia, which concerns reading. What are the causes? Are there remedies? Cerebral imaging can not only help understand how the brain learns, or fails to learn, but also contribute to developing pedagogical strategies suited to these children in difficulty.

"La Leçon" (the lesson) a painting by Pierre-Auguste Renoir in 1906. Although humans are not the only species who learn to communicate and use tools, they are the only ones who teach their young, first of all within the family and then at school. Learning to read consists in establishing a correspondence between a visual form and the sound it represents.

The flexible yet constrained setting of a structure as complex as the brain, inevitably means that something will sometimes go wrong. Consequently, about 5 to 12% of children in a given age class suffer from deficits in a **cognitive** field: dysphasia (spoken language difficulties), dyslexia (reading difficulties), dyscalculia (calculation difficulties), dyspraxia (motion difficulties), hyperactivity or attention deficit, etc. This is not just the psychologists' latest obsession and the percentage of young people experiencing difficulties during the tests carried out for the French National Defence Preparation Days(1) confirms the prevalence of learning problems in France. For example, in 2006, of the 800,000 young people tested, only 78.7% could read effectively. Of the remainder, 9.6% were mediocre readers who read too slowly, 6.9% did not understand a written text and 4.8% could be considered as illiterate. These results match those of the 2009 national assessments in French primary schools, where 73% of the children were good readers, 18% had rudimentary reading skills and 9% were experiencing serious difficulties. In an increasingly technical society, these learning difficulties, which had for a long time remained

(1) Every year, since April 2000, young French men and women aged 17 or more take part in a "National Defence Preparation Day" (since 2010 called the "National Defence and Citizenship Day") during which their reading skills are assessed.

(2) According to Insee's 2007 employment surveys, unemployment concerns 37% of adults with no diploma, as opposed to 9% of adults with higher training.

unknown, create educational problems that seriously jeopardize the professional future of the children[2]. This is why they are increasingly attracting the attention of parents, educators and physicians. The quarter of the population experiencing reading difficulties obviously does not exactly match the specific learning difficulties defined in the medical books. The nosographic[3] categories could appear to be too narrow by comparison with the problems experienced in the field. All the more reason to understand how the brain learns, in order to develop a new pedagogical approach.

Childhood and learning

In all civilisations, humans use the first years of life of their children to teach them, first of all within the family and then at school. We take advantage – not necessarily knowingly – of the considerable **neural** plasticity of the young brain (see *The plasticity of the brain, a still under-estimated capability*, p. 36), to learn how to talk, read, count, play music and so on. Why is it so easy to learn the piano or a second language during childhood, while adults can reason more profoundly and handle far more complex concepts? Why, however, do some obviously intelligent children remain incapable of reading the word "daddy" at the age of 12, or of understanding that if you remove the first sound from "farm" you are left with "arm" (orally), or cannot "see" that 25 is greater than 20 (see *Dyscalculia, the lost meaning of numbers*, p. 34)? These difficulties can persist into adulthood: I have experienced these last two examples in adults, one a driver and the other a doctor of history, who had no problems other than their deficit restricted exclusively to speech or to numbers – a deficit which had made their entire school lives a nightmare.

Genetics no doubt plays a role in most of these difficulties. Numerous **genes** express in the brain of the fœtus, sometimes for short periods and in specific regions, controlling the multiplication and migration of **neurons** and encouraging the right connections. These genes can act directly or can regulate other genes, themselves acting directly or influencing other genes in turn, creating complicated cascades of interaction. Whatever the case, a dyslexic parent has a 50% chance of having a dyslexic child. Numerous genes would appear to be linked to this pathology, but all those identified so far are involved in the migration of neurons. *Post mortem* studies of dyslexic patients have shown clusters of neurons which have usually gone past their target. These incorrectly positioned neurons therefore fail to perform their work correctly. But what work? The relationship between these **cortical** anomalies and the cognitive deficit is hard to establish, even though dyslexia is the most widely studied and best understood of the development troubles.

Environmental influences

Are genes enough to explain the pathology? Clearly not. The environment plays a crucial role in cognitive development. For example, if a twin is dyslexic, his "identical" twin will not necessarily also be dyslexic, even if he is at higher risk than another sibling or a person without a dyslexic parent. Reading difficulties are also more frequently encountered in people from disadvantaged backgrounds. If you have a poor vocabulary or books are not a familiar sight in your environment, you will give up more quickly when faced with the difficulty of deciphering words and you will read less. Reading is just like tennis: the less you practice the less you improve.

Another example of the influence of environment on learning how to read is the "opaque" or "transparent" nature of the language. "Opaque" languages, such as English, do not transcribe speech regularly, unlike Italian, a "transparent" language in which each letter corresponds to a sound and each sound to a letter. In English, the same letters can be read in different ways. This opacity has a price, even for children without problems. At the end of First Grade, Italian children know how to read, while English children will need a further three years before reaching the same level. French is an intermediate language, which is far harder to write (the sound "o" can be written in many ways *o, au, eau, aut,* etc.) than to read ("*eau*" is always pronounced "o"). The prevalence of dyslexia is therefore, unsurprisingly, higher in English-speaking countries. So overall, if the genetic context (usually linked to multiple genes) can contribute to learning difficulties in a given field, the environment also plays a part. It can amplify these difficulties by failing to provide the necessary support or can correct them by utilizing a variety of means of learning.

PHOVOIR

Thanks to their neural plasticity, children learn to play music or speak a second language far more easily than adults.

The contribution of imaging

Can cerebral imaging open up new avenues for education and re-education? (see box on *The meaning of cerebral images*, below). Although the brain is plastic, there are nonetheless certain constraints on its organization: it is impossible to learn everything and certain rules have to be followed. Some pedagogical strategies work, while others do not. Those which adapt to the constraints of the brain are more effective than those which run contrary to our natural

(3) Nosography describes and classifies disorders and diseases.

The meaning of cerebral images

Imaging reveals no "biological" effects that can be assumed to be "fixed". It presents the state of the brain, a plastic organ if ever there was one, at a given moment in time. This state is just as much the product of its biological history as of its cultural environment. Illiteracy and dyslexia can therefore be shown by the same images. Yet one is linked to non-attendance of school and the other to difficulties in learning to read. Imaging is therefore just as capable of exploring the biological causes of dyslexia as the impact of any given educational strategy. This lack of specificity also creates difficulty with interpreting results when comparing a pathological group with a control group, because the observed differences can be linked to the cause of the pathology, or may simply be the consequence of it. For example, if dyslexic subjects do not activate the "visual word form" area, is poor organization of this occipital area the source of the reading problems or, conversely, was automatic letter recognition in this area unable to take place owing to the subject having read an insufficient number of texts? Cause or consequence? It is often hard to separate the two.

tendencies. For example, recognizing faces is obvious for a human and difficult for a computer. On the other hand, multiplying numbers requires an effort on our part while it is a basic operation for a computer. The aim is therefore to find the most effective strategies, taking account of the characteristics of the brain. The first step is obviously to understand how the child or adult with no problems reads, speaks and calculates. So what have we learned from imaging on this subject in recent years? Dyslexia, the most widely studied pathology, is the clearest example of what we can understand. Learning to read consists in establishing a correspondence between a visual form and the sound it represents. This process significantly modifies the visual regions of the brain, which develop a specific response to the strings of characters frequently encountered in coding the sounds of the language. This region, known as the "visual word form" area is situated in the left-hand fusiform **gyrus**, with coordinates that are surprisingly similar from one individual to another, whether or not they are speaking (and writing) the same language (figure 1). This level of reproducibility could appear surprising for a cultural activity, but it is probably linked to the special relationship between this area of the brain and, on the one hand, the visual areas which code information in the centre of the visual field (**fovea**) and, on the other hand, the oral language areas. This word-specific activation is established early on because MRI studies carried out at **NeuroSpin** show that children at the end of First Grade have already developed this response. Reading also significantly modifies the spoken language neuronal network, because it requires analytical awareness of spoken language down to its most basic building blocks, the **phonemes**. Even though, from birth, infants can discriminate phonemes in a way similar to adults, the conscious manipulation of these elements only becomes effective once reading has been learned. This is proven by the poor performance of illiterate subjects in tasks in which they are required to "play" with phonemes.

The dyslexic brain

In dyslexic subjects, the "visual word form" area is activated far less (if at all) during reading tasks than in good readers of the same age (figure 2), whatever the language. Dyslexics also show less activation in the oral language areas and a number of structural studies have revealed anomalies in the left-hand **white matter**, in an area through which the arcuate fascicle (an important bundle of nerve fibres linking the various linguistic regions) passes. These results coincide with the current dyslexia model which attributes reading difficulties to problems with decoding the sounds of speech. The lesser activation of the "visual word form" area would thus appear to be a consequence of the lack of automation of visual word recognition. The difficulty in perceiving the difference between the sounds "b" and "d" clearly makes it impossible to assign letters to them. In addition, for well-known words, the activation in the "visual word form"

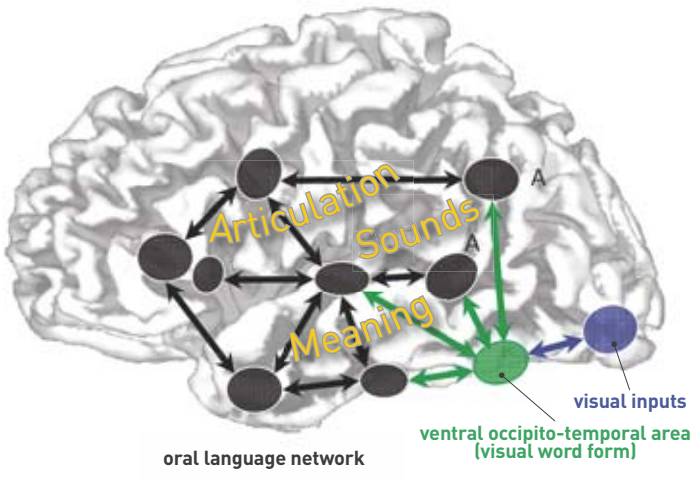
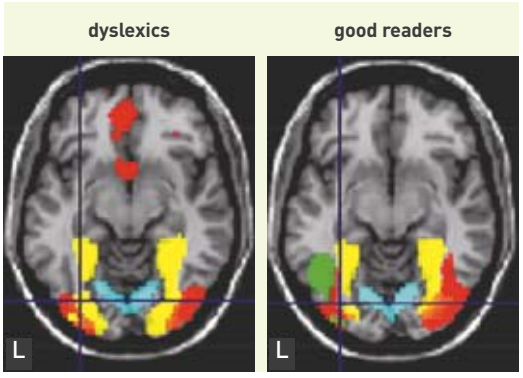


Figure 1.
The cerebral reading circuit. Reading consists in connecting the visual regions (in blue) with the language regions (in black). As of the first year of learning to read, an area known as the "visual word form" area (in green) specializes in letter recognition. This area has trouble in forming in dyslexic subjects because they usually have difficulty in coding the sounds of speech in the posterior temporal regions (A) and in learning the sound/letter correspondence.



CEA – Inserm

Figure 2.
Brain activity in children, both dyslexic and not. The visual region activates in the same way in 9-year-old dyslexic and good-reader children when they look at a checkerboard (in blue), houses (in yellow) or faces (in red). However, looking at written words only activates the "visual word form" area (in green) in good readers.

CEA – Inserm

Girl volunteer taking part in a functional MRI study at NeuroSpin, designed to shed light on learning mechanisms.

area is similar in good-reader and persistent-poor-reader adults. However, these latter also utilize the right prefrontal area, suggesting that they call on memory far more than good readers. As with differences between readers of different languages (see box on *Languages and imaging*, opposite), it is therefore possible to compensate for difficulties by using additional areas of the brain.

Alternative strategies

These observations are clear evidence of the resources at the disposal of the brain, even in case of difficulties, but new strategies then have to be adopted. For example, writing the shape of the letter while speaking its sound and visualizing it, significantly helps with learning the sound/letter correspondence in good-reader children. In dyslexic subjects, using imaging at NeuroSpin, we have observed that those who do best are those who present the most activation in the region corresponding to the hand when they hear speech. This result suggests that they use manual coding to compensate for their auditory difficulties. Such a strategy, which these children no doubt discovered spontaneously, could be systematically and explicitly put to good use.

The evolutionary success of the human species rests on its cultural development, in which each generation teaches the next one. Even in newborns, even dyslexic or dyspraxic, the brain is a wonderful learning machine. If usual teaching methods are not appropriate for a particular child, it is up to us to find a new solution calling on other skills in order to get round the difficulty. The more we understand the brain's mechanisms, the more we will be able to imagine such solutions. Cerebral imaging thus becomes a precise and valuable tool in this quest. Research in this field is only just beginning, because these techniques are still recent. However, the next few years should see an increasingly detailed dissection of the learning mechanisms and the appearance of new pedagogical concepts to avoid allowing one quarter of our fellow citizens to fall by the wayside.

Languages and imaging

The differences between "opaque" and "transparent" languages are visible in imaging. English-speaking readers call far more on the speech production areas, while Italians tend more to activate a more direct channel for storage of speech sounds. To determine the pronunciation of a string of letters, English-speakers are required to call on the frontal areas in the same way as young readers, while this step is not necessary in a regular writing such as Italian. This example shows how different cultural environments can call on varying numbers of highly specific areas of the brain.

> **Ghislaine Dehaene-Lambertz**
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

Dyscalculia, the lost meaning of numbers

Developmental dyscalculia, an arithmetical learning disorder, originates from an alteration in the "meaning of numbers". Humans – like other animal species – have an innate capacity to distinguish quantities. The neuronal basis for this lies in a region of the cortex that is also involved in spatial awareness. These are all avenues for the development of early diagnosis tests and tools for re-educating children suffering from dyscalculia.

wavebreakmedia/Shutterstock.com

Developmental dyscalculia, or arithmetical learning difficulties, affects children whose intelligence is otherwise perfectly normal.

Some children, even though normally intelligent, are unable to solve operations as simple as 7 - 3. Others are unable to estimate or compare quantities with the naked eye, even when only faced with two or three objects. They have trouble understanding that one number can be greater than another and in linking different types of numerical symbols (spoken numbers, numbers written with Arabic numerals, etc.). A number of studies published in the United States and Israel estimate that 5% of children experience unusual difficulties in learning arithmetic. This trouble is called *developmental dyscalculia*. It is similar to dyslexia, which is manifested by difficulties in learning to read, but is far less well-known and understood (see *The contribution of neuroscience to learning: the example of dyslexia*, p. 30). In just the same way, it can occur in children with a normal or above-average IQ, living in a social and family environment with no particular problem. Like dyslexia, dyscalculia is sometimes associated with other **cognitive** deficits which, in this case, are typically spatial in nature (problems with spatial orientation, motor difficulties, spatial memory, etc.).

Where does dyscalculia come from?

It is still hard to give a definitive answer to this question. On the one hand because there are no doubt several sub-types of dyscalculia, and on the other because the lack of any common diagnosis test means that the results obtained in different centres and different countries cannot be systematically compared. However, current results suggest that the main cause of dyscalculia is a primary trouble in the perception of numerical quantities which, during learning, leads to difficulties in understanding numerical symbols (Arabic numerals) and the principles of arithmetical calculation. Neuroimaging shows that this trouble is linked to a disorganization of the **neurons** in the interparietal region of the **cortex** which, in macaques and in man, is home to neuronal populations dedicated to the representation of the cardinality(1) of a set of concrete objects and the mental transformation of cardinalities (additions, subtractions, etc.). These neurons are known as the "number neurons" (figure). In humans, this specialization of the parietal cortex for numbers is apparently an evolutionary inheritance(2).

Impaired number sense

An example of this intuitive capacity: if we let five objects fall into an opaque jar and then add five more, an infant will spend longer looking at the final scene if, once revealed, the jar only contains five objects instead of the expected ten. His innate "number sense" has warned him of a calculation error. Later on, during learning, children understand numerical symbols (names of numbers, Arabic numerals) and formal calculation because their brain is already capable of representing numerical quantities and

(1) Cardinality: property of a set representing its "size" and thus the number of its elements.

(2) Many animal species possess astonishing mathematical capacity, enabling them to differentiate between the number of objects in two sets, but also to carry out approximate calculations.

their transformations. This intuition thus gives quantitative meaning to symbols which would otherwise remain meaningless. In the brain, we have demonstrated that it is precisely the regions of the parietal cortex representing sets of concrete objects which are activated when we perform mental arithmetic with numbers!

It is then clear that an early disorganization of these cortical circuits can lead to a selective loss of number sense and to difficulties with mathematics. In children suffering from dyscalculia, number sense is impaired: they have trouble discriminating between two sets of objects and in estimating the result of an arithmetical operation between these sets. This troubled numerical perception is the cause of the difficulty they experience when comparing Arabic numerals or carrying out mental arithmetic. Imaging shows a structural alteration in their parietal cortex (smaller volume of **grey matter**, modified pattern of cortical **gyri**) and **metabolic** changes by comparison with children with no dyscalculia problems.

Weakened spatial capabilities

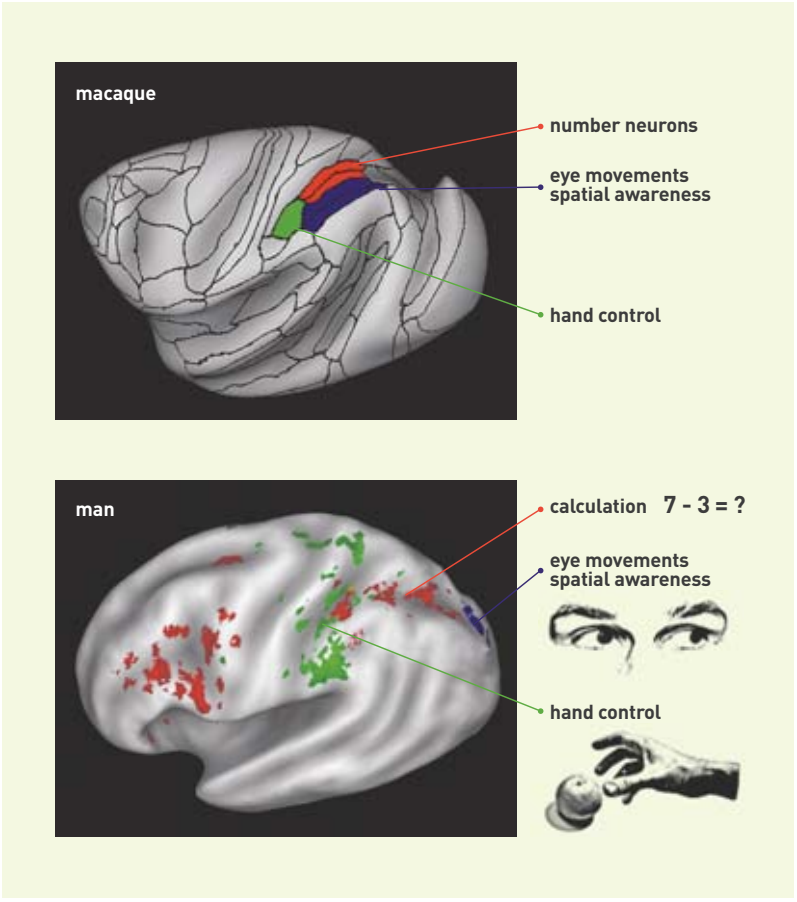
The neuronal circuits dedicated to numbers are part of a mosaic of parietal cortex regions assigned to spatial processing and involved in controlling spatial awareness and fine-tuned programming of movement (figure). These cerebral regions specializing in the processing of spatial and numerical information develop together and their extent in adults shows a strong **genetic** correlation. This suggests that the frequent association between dyscalculia and troubles with spatial orientation (as well as with time) and fine-tuned movement control (as in certain cases of dyspraxia) has a neurobiological basis.

The genetics of dyscalculia

There is most probably a genetic contribution to dyscalculia, because its risk of occurring within the same family is 10 times greater than its occurrence in the general population. In monozygotic (identical) twins whose genetic heritage is rigorously identical, if one is affected, the other will also be affected in 70% of cases. Moreover, dyscalculia is often – but not always – present in pathologies of genetic origin, such as **Turner's syndrome**, **fragile X syndrome** or **Williams' syndrome**. Environmental factors also have a strong impact, in particular during the early brain development phases. Dyscalculia is thus more frequent in premature newborns and in those exposed to alcoholic intoxication by their mothers during the foetal period.

Diagnosis and re-education

Developmental dyscalculia is a trouble of neurobiological origin, unrelated to poor teaching, laziness or any lack of motivation on the part of the child. It is not rooted in emotional or relational problems, as was suggested by some psychoanalytical orientation therapists in the past. It is therefore essential to identify this problem as early as possible to avoid heaping guilt on the child, thus preventing a loss of self-confidence and unnecessary psychological suffering. Applied research is today being mobilized to develop early diagnosis tests and to design new re-education tools, the efficacy of which will need to be assessed experimentally. This is the case with two tools taking the form of computer games, which we created in the Unicog laboratory at **NeuroSpin**: "*La Course aux Nombres* (The Number Race)" and "*L'Attrape-Nombres* (The Number Catcher)"[3]. These games are designed to reinforce early intuition of numbers and connection with the sense of space and the recognition of words and numerals.



CEA – Inserm

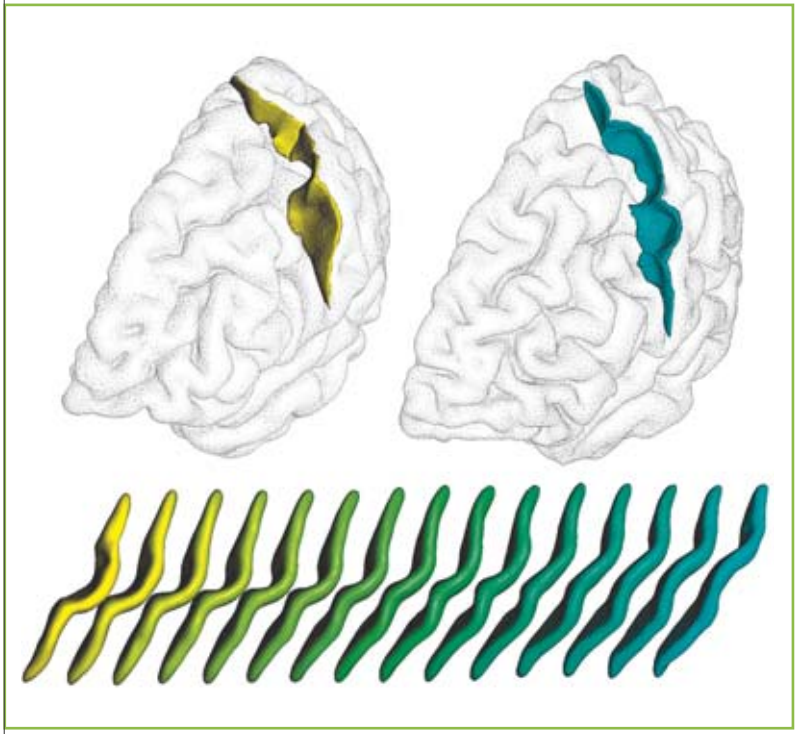
Figure.
At the top, cerebral regions of the parietal cortex involved in processing numbers and space in macaques. At the bottom, cerebral regions of the parietal cortex involved in calculation and spatial processing in humans.

> Manuela Piazza
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

(3) http://www.thenumbercatcher.com/ and http://www.thenumberrace.com/.

The **plasticity of the brain**, a still under-estimated **capability**

Unlike a computer, our brain is not "hardwired" once and for all at birth, but evolves with experience. Learning to read is the perfect illustration of this cerebral plasticity. MRI reveals to what extent this learning and, for example, the situation of frustrated left-handers, significantly remodel the anatomy of the brain. These are all lessons that must be taken into account when defining improved pedagogical strategies.



CEA/Z.-Y. Sun, D. Rivière and J.-F. Mangin

Frustrated left-handers: proof of the plasticity of the brain. The shape of the left central sulcus, which houses the sensorimotor areas linked to the right hand, depends on the manual laterality. Left-handers have a bias towards the yellow shape, while right-handers tend more towards the blue shape. Systematically using a single hand to write leads the associated sulcus to lengthen, even though its overall shape does not change. Consequently, frustrated left-handers resemble left-handers in the shape, while the lengths of their two central sulci show the same asymmetry as that of right-handers.

At birth, the human infant possesses some one hundred billion **neurons** which will no longer multiply, except in a few specific regions such as the hippocampus. Its brain is not however fully developed, far from it, because the connections between these neurons, the **synapses**, are barely starting to form. Only 10% of them are present at birth. They will rapidly become one million billion, with a selection process eliminating the least active. About twenty years will be needed for cerebral connectivity to reach full maturity. This plasticity – this is the name of this capacity of the brain to remodel itself according to what it experiences – will then diminish, although without completely disappearing. The brain is not therefore hardwired once and for all, like a computer. We are able to learn new things throughout our lifetime.

Converting an existing structure

The idea that the brain is a blank slate on which any type of learning can be written is however completely incorrect. In fact, this learning process uses machinery that is already in place, albeit with a degree of flexibility. From the outset, the brain is already organized to be able to smell, speak, hear, see… For example, it is quite possible that there is already a specific system "hardwired" for face recognition. This function, which is vital to social species such as our own, could have been selected during the course of evolution. On the other hand, there is clearly no brain module specializing in reading. This cultural invention is far too recent to be part of any **genetic** brain development "program". The human brain is not therefore "hardwired" to be able to read. It is only the intensive learning of reading itself that will mobilize numerous neural circuits, which will then be subjected to constraints leading to a remodeling of the existing wiring. The acquisition of reading thus converts those neural networks initially dedicated to the visual recognition of objects and enables them to identify a written word in just a few milliseconds (figure). After a few months of learning, this phenomenon becomes visible with functional Magnetic Resonance Imaging (MRI)[(1)].

The effects of reading

Learning to read at such an early age, at a time when the plasticity of the brain is at its peak, is certainly no hazard. By immersing children in an artificial environment of letters and words, we no doubt reorient a good number of neurons in their inferior temporal **cortex** so as to optimize how they code writing. The acquisition of reading can also lead to an even more impressive plasticity. The learning of braille by visually impaired children modifies the

(1) The identification of written words does not call on the face recognition area of the brain. This latter records things "globally" while visual recognition of objects is relative to spatial topology.

structure of their brain: areas specializing in touch invade the regions normally involved in vision. This potential for remodeling persists into adulthood but significantly decreases, hence a far more laborious learning process. All these recent results once again come from functional MRI observations.
There is also virtually no doubt that the hours spent recognizing tiny differences between characters increases the analytical skills of our visual system. A comparison with illiterate subjects in fact reveals that the perception of geometrical forms improves with learning to read. These discoveries on the cerebral effect of learning to read demonstrate that the highly specialized regions of the brain are not necessarily innate: they can be the result of learning.

MRI, or how to see plasticity

The plasticity of the human brain is therefore still under-estimated. Although it would be absurd to deny the existence of genetic constraints on brain capacity, one can however only consider them, at best, as facilitating the learning of certain functions, by giving each sector of the cortex specific qualities and drawbacks. It is also probable that these properties are more the result of the connectivity of a sector with the rest of the brain than the specificity of the local microscopic architecture.
Functional MRI is not the only imaging technique available for studying the plasticity of the brain. Diffusion MRI, which relies on the random movements of water molecules in the brain's tissues, can reveal microscopic modifications in the cabling of the brain, following intensive learning. With clinical MRI techniques, it is now common to be able to detect macroscopic modifications in the morphology of the brain induced by a learning process. We thus recently observed that literate persons, by comparison with persons who have never learned to read, show better organization of a bundle of connections in the left hemisphere, probably involved in the transmission of visual information to the language areas. Even persons who became literate in adulthood show this change, which clearly demonstrates that the brain remains plastic throughout our lifetime.

Cerebral body-building

The effects of this cerebral body-building can be seen even as far as the shape of the **gyri** of the cortex. For example, the central sulcus, which houses the sensorimotor areas, is more marked in the dominant hemisphere: that of left-handers is longer in the right hemisphere, while that of right-handers is longer in the left hemisphere (see illustration p. 36). This asymmetry is quite possibly linked to the origin of manual laterality. There are also other morphological differences between left- and right-handed people: the central sulcus forms a bend during embryonic development, which is found to be lower in the left hemisphere in left-handers than in right-handers. A study of frustrated left-handers showed that, just like in left-handers, this bend remains lower than in right-handers. Yet, forced training of the right hand to write led their left sulcus to extend until it was as long as that of right-handers. It is therefore quite probable that the asymmetry in favor of the dominant hemisphere in the entire population is the result of the long hours spent writing at school. Finally, experience does not simply modify the synaptic connections of the brain: it can even modify the folds of the cortex. This is no doubt a means of regulating the changes in tissue volume due to the microscopic modulations.

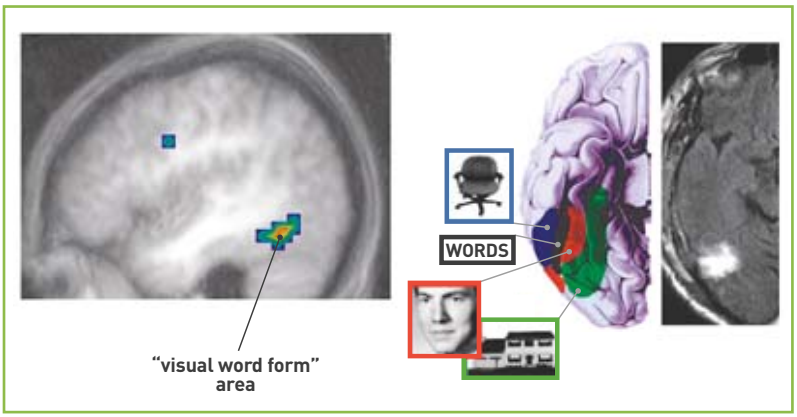


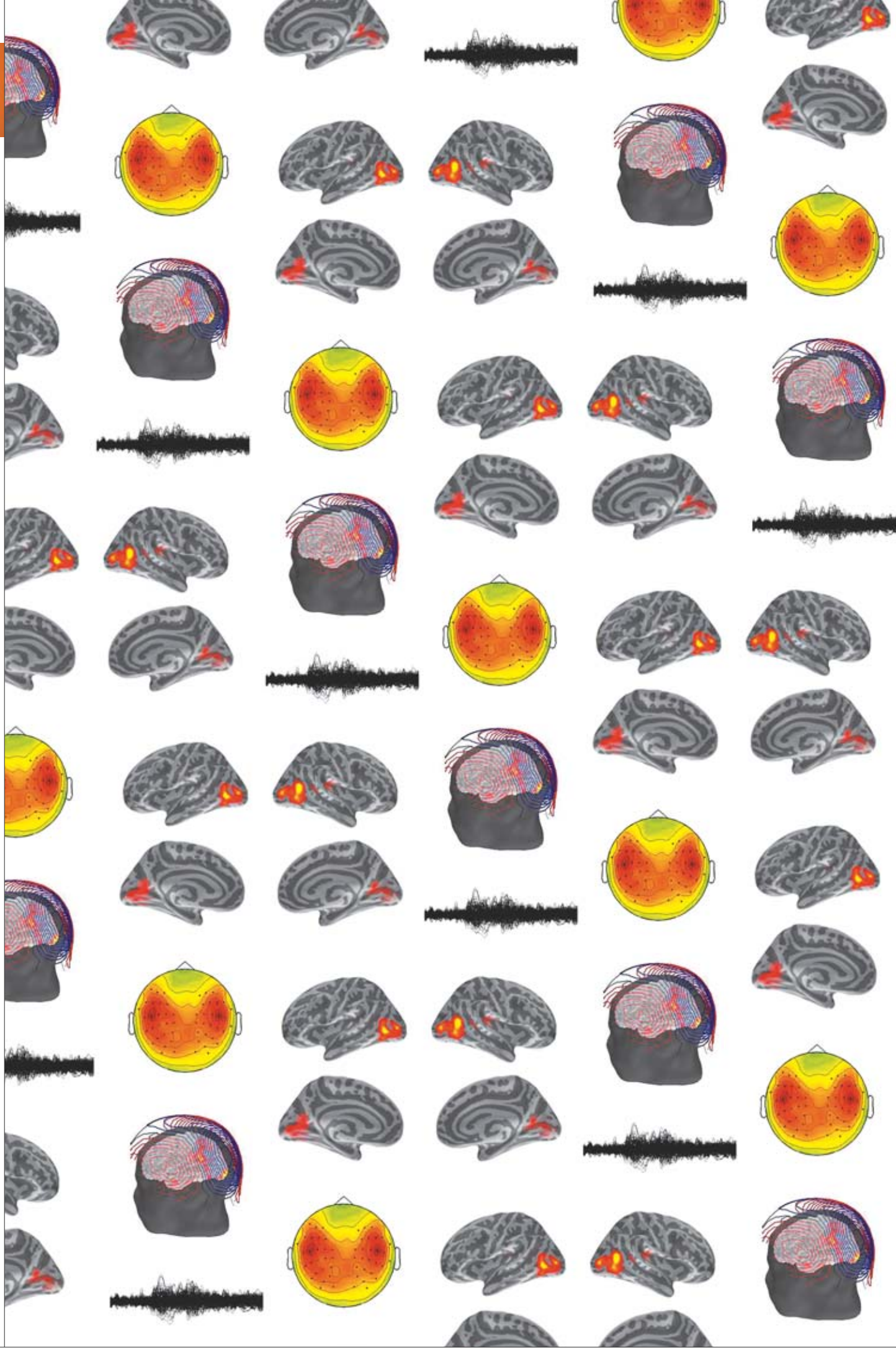
Figure.
The "visual word form" area – the brain's "letter box" – is a small region of the human visual system that systematically activates whenever we read. It shows a stronger activation to words than to many other categories of visual stimuli, such as pictures of objects, faces or places. In all of us, it is systematically located at the same place within a "mosaic" of ventral preferences for various categories of objects. And, if it is destroyed or disconnected, as in the patient whose brain scan is shown at right, we may selectively lose the capacity to read.

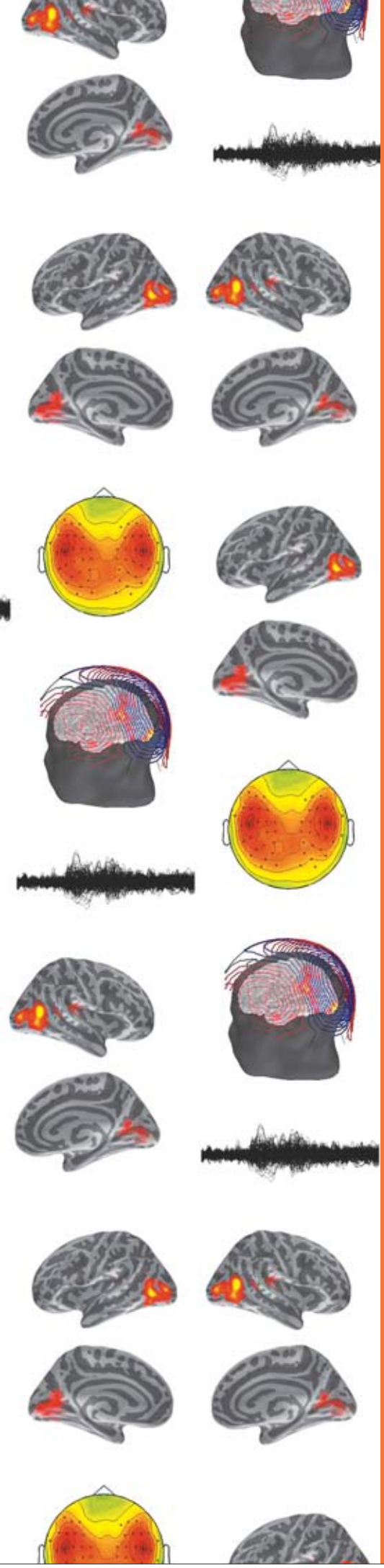
How do we learn to read?

This progress in our understanding of the plasticity of the brain encourages a degree of optimism. Thanks to its extraordinary powers of adaptation, our brain is able to brilliantly take on board the never-ending changes in our environment. Although this plasticity diminishes with age, it remains present, as witnessed by the growing success of re-education techniques employed following strokes. Some educational choices would also certainly benefit from a better understanding – and application – of the plasticity of the brain. The constantly recurring debates on the ideal strategy for learning to read would for example become less heated…

> **Jean-François Mangin**[1]
and **Stanislas Dehaene**[2,3]
Institute of Biomedical Imaging (I2BM) – NeuroSpin
[1]Information Analysis and Processing Unit
[2]Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

[3]Collège de France, *Paris*
and University Paris-Sud 11, *Orsay*





Responses recorded by MEG when the subject sees and/or hears stimuli.
CEA/NeuroSpin

III. COGNITIVE ARCHITECTURE AND THE BRAIN

The complexity of information processing carried out by the brain is gradually being revealed by experimental studies combining functional neuroimaging with cognitive neuroscience and computational science. The information encoding and decoding operations performed by the brain are illustrated here by three main cognitive functions, that are language, consciousness and temporal cognition.

Let us take the example of language. Understanding a sentence does not simply mean understanding the words it contains, but also its structure, *i.e.* its syntax. We are exposed on a daily basis to new sentences which we have never read or heard, yet that we are capable of analysing and making sense of. This is possible because our brains have areas specializing in the processing of hierarchical structures called syntactic trees. The discovery of this hierarchical pathway within the brain by means of cerebral imaging marked the beginning of research into a neural model for language processing.

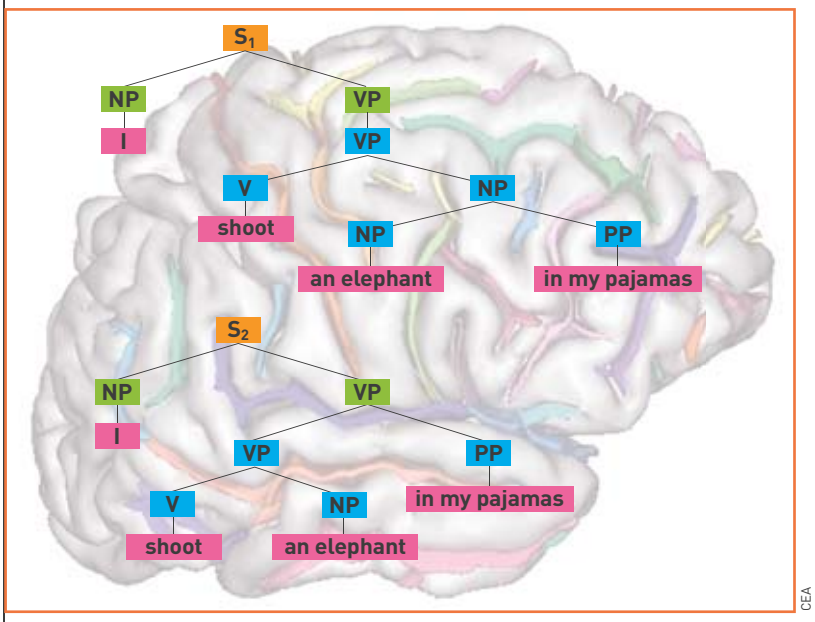
The researchers have also recently discovered that in order to be consciously perceived, a visual stimulus such as a word must induce neural activity which gradually extends to a large number of brain regions and which persists more than 300 milliseconds after the presentation of the word. If the brain's activity remains focal and of short duration, the word will not be consciously perceived. This characterization of neural activity can be used to detect consciousness disorders: it is sufficiently discriminating to be able to identify the state of consciousness of an individual in a coma.

Temporal cognition, which includes the perception of time, is another fundamental characteristic of the human being. The time perceived is more than just the simple physical timeline and is enhanced by other mental properties such as anticipation, causality or duration; all these subjective representations contribute to our mental representation of time. The numerous data that reach the brain on various time scales must be integrated in order to produce what we call our time consciousness.

> Virginie van Wassenhove
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

Syntactic structures and the brain

The understanding of language, a faculty specific to the human species, involves many more mechanisms than simply recognizing words. To correctly interpret a sentence, particular account must be taken of its grammatical structure (or "syntax"). Non-invasive cerebral imaging enables us to find out about the activity of the brain when it groups words in these syntactic structures.



CEA

Figure 1. Examples of syntactic trees. A given sequence of words can be associated with different structures and interpretations; NP, VP and PP respectively signify Noun Phrase, Verb Phrase and Preposition Phrase.

Like many animal species, we have an impressive ability to identify "objects". We can easily recognize the noise of a train, the silhouette of an animal in a landscape, a perfume and so on. This apparently banal capability is due to cerebral systems which match the sensorial inputs with representations stored in the memory – and in so doing resolve perceptual invariance problems that go beyond the capabilities of the best automatic shape recognition systems.

There is however one field where memory, no matter how efficient, is no longer enough: language. We are regularly exposed to sentences we have never heard, and yet we understand them with no problem. Our brains master language and can thus reconstruct the meaning of an unknown sentence based on the words it contains. Which of the brain's circuits are responsible for the operations underpinning this faculty, apparently specific to humans?

The organization of sentences

Language can be carried by writing, speech, sign language, or even more exotic media such as the whistled language of the Canary Island shepherds. In any case, after initial processing by the primary sensorial visual or auditory area, the sentences activate areas of the brain located primarily in the left hemisphere, along the superior temporal sulcus, as well as in the inferior frontal **gyrus**, better known as "Broca's area".

A part of this network, the epicenter of which would appear to be situated in the middle of the superior temporal sulcus, seems to be involved in recognizing and understanding words. However, to interpret a sentence, it is not enough to understand the words it contains. One must also identify the relationships between them, for example to determine the subject and object of a verb. These relationships can be expressed by the positions of the words or by grammatical markers (such as case declension marks in German). In French, in a declarative sentence, the subject appears before the verb which precedes the object; in other languages, such as Japanese, the verb is at the end, after the object. Another characteristic of human languages is that the sentences can be embedded, *i.e.* they can themselves contain other sentences. For example, the following sentence: "The car that my father bought yesterday has broken down" contains a relative clause.

Neural coding of syntax

To describe the relationships between words and the phenomena of embedding, most linguistic theories postulate that sentences are mentally represented by hierarchical structures, also called "syntactic trees" (figure 1), which organize their component parts. An active branch of neurolinguistic research focuses on understanding how these structures are encoded in the brain. A Magnetic Resonance Imaging (MRI) experiment conducted by the teams at **NeuroSpin**, was thus carried out to identify the areas of the brain involved in calculating the syntactic structure. The starting point for this work was a result obtained by the American theorist Paul Smolensky, who proposed an artificial **neural network** model to represent tree structures. This model goes on to make what is in fact a relatively simple prediction: the more complex a coherent group of words – as opposed to a string of disconnected words – the greater the activation of the network which encodes it. The complexity of such a group, also called the "constituent", is quite simply measured by the number of words it contains.

condition	examples of stimuli [real words/pseudo-words (Jabberwocky)]											
c12	I	believe	that	you	should	accept	the	proposal	of	your	new	associate
	I	tosieve	that	you	should	begept	the	tropufal	of	your	tew	viroflate
c06	the	mouse	that	eats	our	cheese	two	clients	examine	this	nice	couch
	the	couse	that	rits	our	treeve	fow	plients	afomine	this	kice	bloch
c04	mayor	of	the	city	he	hates	this	color	they	read	their	names
	tuyor	of	the	roty	he	futes	this	dator	they	gead	their	wames
c03	solving	a	problem	repair	the	ceiling	he	keeps	reading	will	buy	some
	relging	a	grathem	regair	the	fraping	he	meeps	bouding	will	doy	some
c02	looking	ahead	important	task	who	dies	his	dog	few	holes	they	write
	troking	ahead	omirpant	fran	who	mies	his	gog	few	biles	they	grite
c01	thing	very	tree	where	of	watching	copy	tensed	they	states	heart	plu
	thang	very	gree	where	of	wurthing	napy	gunsed	they	flotes	blart	trus

Figure 2.
The different sequences used in the experiment. The size of the constituents increases from the bottom (1 word c01) to the top (12 words c12).

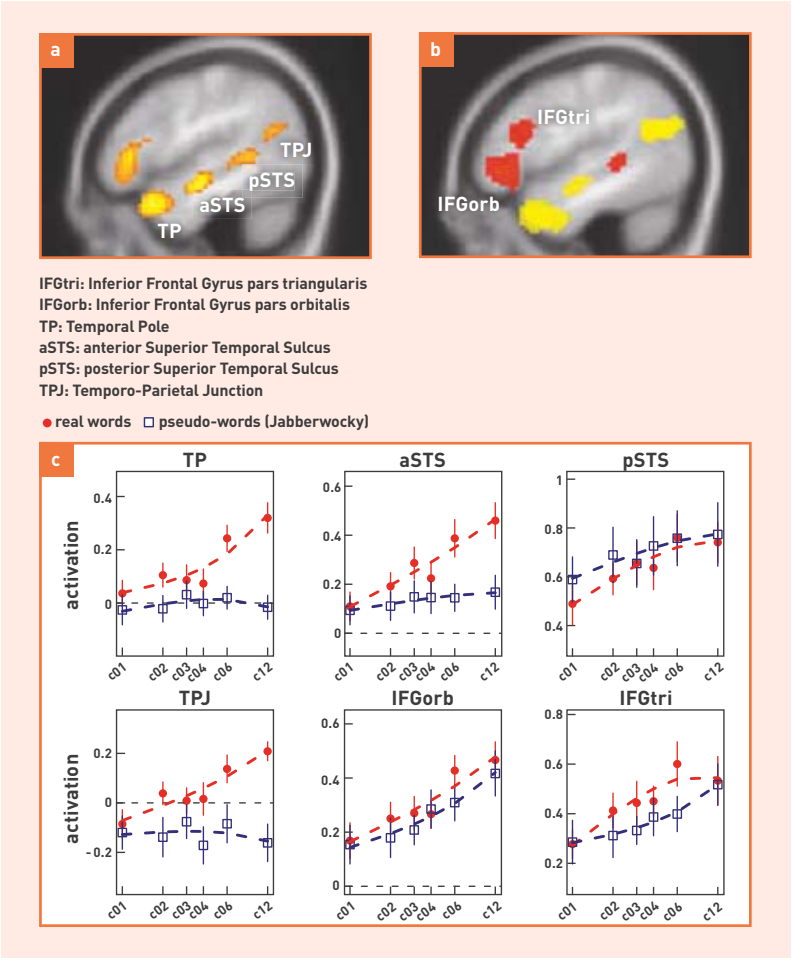
Grammar and MRI

The experience carried out at NeuroSpin used sequences of 12 words organized into coherent constituents in ascending order of size. Some sequences were strings of words unconnected to each other, others comprised groups of 2 words, others 3 words, then 4, 6 or 12 words – in this latter case, the sequence corresponded to a complete sentence. Another set of sequences was constructed in exactly the same way, but the content words – nouns, verbs, adjectives, adverbs – were replaced by pseudo-words (figure 2). This manipulation aimed to reduce the contribution of the meaning of the words, focusing more on pure calculation of the syntactic structure. A constituent such as "the mayor of the city" is thus coherent from both the syntactic and semantic viewpoints; however, "the tuyor of the roty" is syntactically acceptable but evokes no semantic representation.

During the experiment, volunteers in the MRI scanner were shown these sequences and had to read them, word by word. The data were analysed, with the aim of looking for the areas of the brain in which activity was modulated by the size of the constituents. Figure 3a shows the areas in which activity increases with this size when the sequences are made up of real words. A significant part of the language network is then involved. However, with sequences of pseudo-words, only the regions identified in red on figure 3b saw their activity increase according to the size of the constituents. This concerns the inferior frontal gyrus (Broca's area) and the posterior part of the superior temporal sulcus. The activation diagrams (figure 3c) reveal that these regions are virtually insensitive to the lexical status, *i.e.* to whether the sequences contain real words or pseudo-words.

Contribution of imaging

These results reveal a number of important facts for a neurolinguist. On the one hand, they show that grammatical words (definite articles, pronouns, etc.), as opposed to content words, are enough for certain parts of the brain to be able to construct a syntactical representation of the sentence. On the other, they shed light on the quantitative relationships between the size of the constituents and the activity in several areas, information which is useful for fine-tuning theoretical models of structure encoding in neural networks. We are still a long way off understanding the neural code of syntactic structures, but this experiment shows how non-invasive cerebral imaging techniques can help.



CEA – Inserm

Figure 3.
Results of the MRI experiment aiming to identify the areas of the brain involved in the calculation of the syntactic structure. In a, the regions where activation increases with the size of the constituents in sequences of real words. In b, result of the same experiment with sequences of pseudo-words (Jabberwocky). Only a part (in red: pSTS, IFGtri, IFGorb) of the previously identified regions responds in the same way as with real words. In c, activation of the regions according to the size of the syntactic constituents (red = real words; blue = pseudo-words).

> Christophe Pallier
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

Towards **scientific exploration** of **consciousness**

How do we perceive our environment? How does the brain create a conscious representation of an object? These questions have been troubling researchers since the dawn of neuroscience. Progress in cerebral imaging and in experimental psychology means that it is now possible to scientifically explore the neural mechanisms of consciousness. Major discoveries have been made recently, some at CEA's NeuroSpin centre.

CEA – Inserm

Volunteer installed in the MEG system and wearing an electroencephalography cap. The EEG and MEG enable information transfers through the brain to be monitored millisecond by millisecond.

Studying conscious perception in humans is now possible thanks to experimental psychology protocols during which a stimulus presented to a participant will sometimes be visible and sometimes invisible. The contrast between these two situations is essential: the sensory stimulation is identical and only the subject's subjective perception is different. For example, when two images are presented in less than one hundred milliseconds (ms), the first is frequently "masked" by the second. The observer has absolutely no conscious awareness of the first stimulus. Cerebral imaging has however revealed that the brain partially integrates the sensorial information: the presentation of the stimulus induces activation in the visual **cortex**, at the back of the brain. When the same stimulus is perceived consciously, the **neural** activity is considerably increased in the visual regions and propagates to the parietal and frontal areas of the brain (figure 1). ElectroEncephaloGraphy (EEG) has been able to measure the time course of this activation. From about 100 to 250 ms after the presentation of the stimulus, it is mainly the visual regions of the brain that are active. Activity reaches the frontal and parietal areas after 350 ms. The particularity of the amplitude of this activation, named "P300", is that it is closely linked to the subjective visibility of the stimulus as reported by the observer.

Psychological refractory period

The brain is limited in its ability to perform several tasks simultaneously. If two stimuli are presented less than half a second apart (for example a telephone which rings while you are driving), the conscious perception of the first will either delay, or preclude the perception of the second. Neural activity in the parietal and frontal regions during this "refractory period" is delayed when the observer is slower in responding to the second stimulus and completely absent when the second stimulus is not consciously perceived. From a behavioural viewpoint, the refractory period

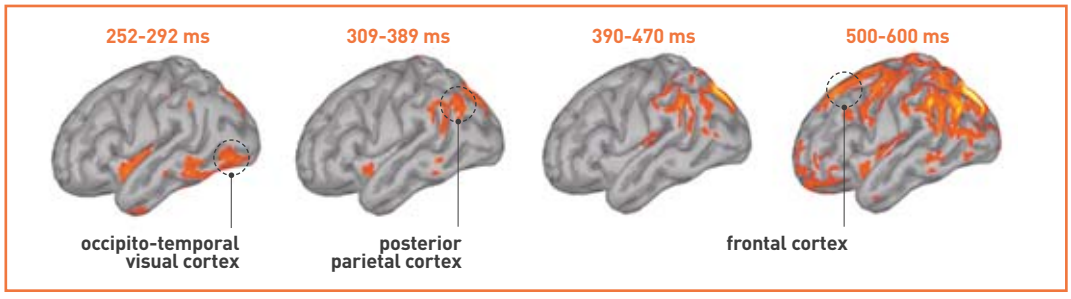


Figure 1.
Cerebral activity measured with MagnetoEncephaloGraphy (MEG) when a letter is "flashed" (one tenth of a second) on a screen. The temporal evolution of brain activity in the left hemisphere, from the visual regions mainly integrating the physical properties of the stimulus (colour, contrast, etc.) to the parietal and frontal regions linked to the observer's subjective perception, is shown from left to right. The time given corresponds to the time elapsed after the appearance of the stimulus.

adapted from S. Marti *et al.*, "A Shared Cortical Bottleneck Underlying Attentional Blink and Psychological Refractory Period", *NeuroImage*, **59(3)** (2012) 2883-2898

PHOVOIR

It is dangerous to answer the telephone when driving, because the brain is limited in its ability to carry out several tasks simultaneously.

creates an illusion in the perception of the second stimulus. Even if both stimuli are presented almost simultaneously, the observer subjectively perceives the appearance of the second as coinciding with the end of processing of the first stimulus. Although the sensorial regions can integrate several stimuli in parallel, activity in the frontal and parietal regions is strictly serial, processing only one stimulus at a time, and closely reflects the subjective experience of the observer.

Cerebral activity and states of consciousness

It is then possible to use the properties characteristic of the neural activity linked to conscious perception as markers of consciousness for individuals in which no subjective report can be measured. For example, a recent study revealed that non-linear activation, similar to P300 observed in adults, was observed in infants, although more belatedly. Between five and fifteen months after birth, the latency of this activation gradually decreases from 900 ms to about 750 ms, which suggests that the same brain mechanisms for conscious access are already present. The same markers were used in an attempt to identify residual consciousness states in patients experiencing chronic disorders of consciousness. The standard neurological examination of these patients is able to classify them in three categories: *vegetative*, *minimally conscious* and *conscious*. In the three cases, the patients are in an awake state but are differentiated in their ability to respond to the environment, show signs of voluntary behaviour and communicate with others. While conventional methods were able to identify a conscious state only in conscious patients, a new method based on the use of decoding algorithms has been able to identify residual consciousness states in 14% of vegetative patients, 31% of minimally conscious patients and 52% of conscious patients. In the same patients, analysis of cortical connectivity revealed that the sharing of information through the brain, in particular between the frontal and parietal areas, increased systematically with the state of consciousness (figure 2).

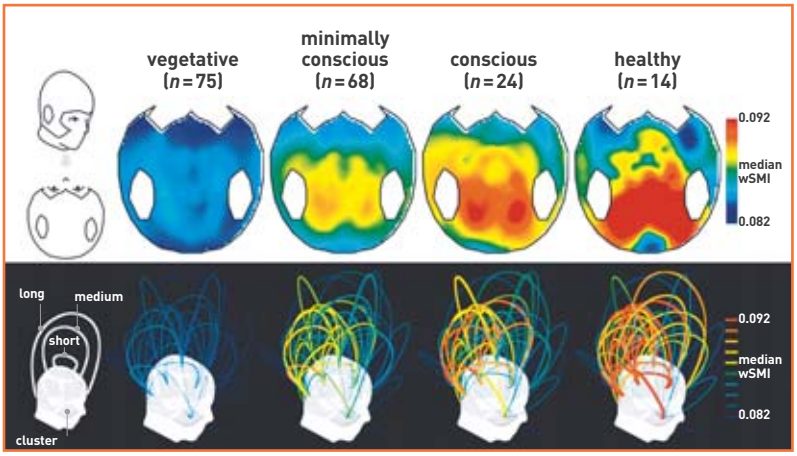


Figure 2.
Cortical connectivity [weighted Symbolic Mutual Information (wSMI)] increases with the state of consciousness. At the top, median connectivity shared by each electrode with all the other electrodes; *n* corresponds to the number of patients included in the group. At the bottom, 120 pairs consisting of 16 clusters of EEG electrodes represented as arcs. The colour and thickness of each line represent the mean wSMI shared by the corresponding pair.

adapted from J.-R. King *et al.*, "Information Sharing in the Brain Indexes Consciousness in Noncommunicative Patients", *Current Biology*, **23**, 1-6, October 7, 2013

Unanswered questions

Research into the neural mechanisms of consciousness is enjoying unprecedented growth. Although experimental discoveries have led to the drafting of theories and models of conscious perception, fundamental questions remain to be answered. Is our perception of ourselves as individuals important in conscious perception? How to show that neural activity reflects the full complexity of an observer's subjective experience? How did biological evolution give birth to the neural architecture necessary for consciousness? These questions will be explored through a combination of experimental psychology, biology and computational science.

Sofie Gelskov

Contrary to a long-held belief, babies have a form of consciousness similar to adults, as of the age of 5 months. The aim of this study, conducted by researchers at NeuroSpin and CNRS, was to determine whether neural markers of consciousness observed in adults could also be seen in infants. The presence of this neural signature of consciousness was tested on 80 infants aged 5, 12 and 15 months, with their brain responses being recorded by EEG.
Here a 5-month-old infant who took part in this study, with his mother.

S. Kouider *et al.*, "A Neural Marker of Perceptual Consciousness in Infants", *Science* 19 April 2013, Vol. **340** no. **6130**, pp. 376-380

> **Sébastien Marti**[1] and **Stanislas Dehaene**[1,2]
[1]Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

[2]Collège de France, *Paris*
and University Paris-Sud 11, *Orsay*

Is time a matter of consciousness?

Philippe Matsas © Flammarion

Étienne Klein

C. Lebedinsky

Virginie van Wassenhove

Étienne Klein: **As it appears on the physical time line, the present instant has no duration: it is concentrated at a point. But our perception of it is never that condensed. Our consciousness expands its duration and places it in its temporal environment, in that it allies the recent past and the imminent future within the perceived present. Our awareness of the present thus collates successive instants which do not in fact physically coexist. Will neuroscience succeed in understanding the temporal "integration" performed by our consciousness?**

Virginie van Wassenhove: The brain is a complex biological system capable of bulk parallel processing of data. When the brain analyses its environment at any given instant, the perceptual information is sent to separate areas of the brain, specializing for example in the analysis of sound (auditory cortex) or the analysis of the identity and location of visual events (ventral and dorsal streams of the visual system). For the brain, an instant can have a duration, a physical period of time during which the information is converted into a neural code.

The time (or moment) scales depend on the temporal sampling used by the neural network in question. For example, the visual system can sample visual information at a frequency of about 10 Hz, while the auditory system is capable of sampling acoustic information far more rapidly (40 Hz). This encoding of information on different time scales in separate anatomical regions would seem to suggest that these instants are distributed and coexist within the brain. However, these instants are not yet necessarily conscious.

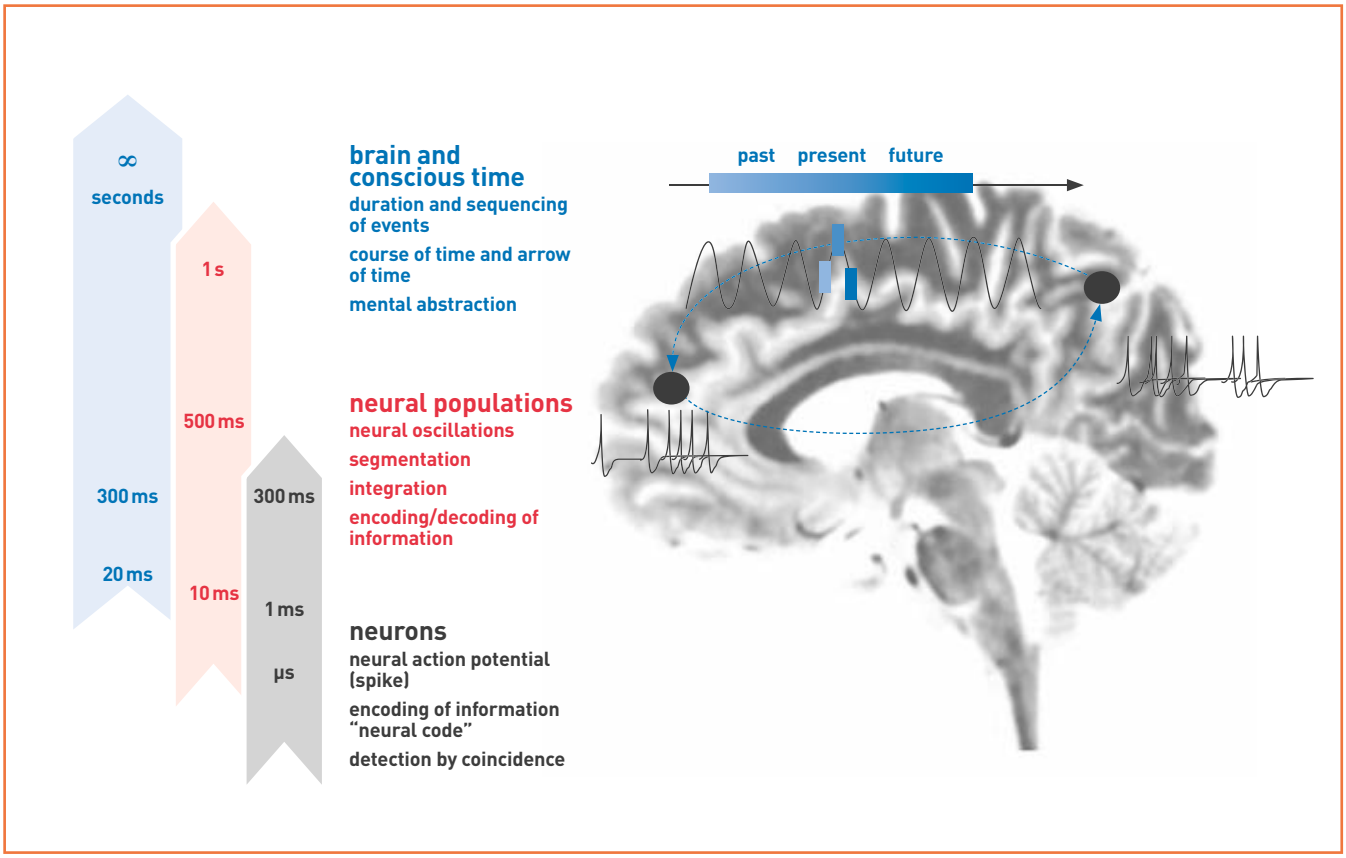
Once these instants have been created, the problem is to bring together the information encoded in the different networks and reintegrate it in a whole that is meaningful to our consciousness. This process takes time – a few tens to a few hundred milliseconds. One working hypothesis is therefore that the duration of the integration process in the network involved in the conscious representation of events is a psychological instant or moment. Phenomenologically, this process is Husserl's "thick present".

ÉK: **We say that time flows or passes. But does it *itself* flow or pass? Or is this just an impression that comes entirely from ourselves? To answer these questions, it must be possible to identify and characterize the "time engine": what is this force which, as soon as a present instant occurs, immediately replaces it by another present instant? Is this time engine physical, or is it linked to us, the conscious subjects? Opting for this second hypothesis, as did certain philosophers (Kant, Husserl, etc.), obliges us to face up to the following, recently revealed facts: the Universe is at least 13.8 billion years old; the Earth was formed 4.45 billion years ago; life appeared on it 3.5 billion years ago and Man only appeared a short 2 million years ago. So what do these numbers bluntly tell us? That objects older than any life form on Earth did indeed exist in the past Universe; that a countless number of events took place, unwitnessed by any human consciousness. So, if the passage of time depends on us, how did time elapse before we appeared? Do you not feel that this "paradox of ancestrality", as it is known, is an impossibility? Is not confining time *in the subject*, or wanting time to have only a subjective reality, the same as refusing to explain the appearance of the subject *in time*?**

VvW: Do the laws of physics require consciousness in order to exist? It is essential to clearly define the problem and differentiate the time of physics from psychological phenomena when talking about the "perception of time". From the viewpoint of cognitive neuroscience, the conscious reality of the world is by definition reduced to the mechanics of the brain. The brain is able to reconstruct the physical reality of what surrounds us, but also of creating parallel realities: for example, to project into the future or remember past moments which are no longer before us in the here and now of the present world. Yet, these mental projections are indeed part of the physical reality of the present!

In neuroscience, there are two major classes of temporal phenomena. The first is that of "implicit time": this concerns biological phenomena which synchronize us automatically with physical time. This is the case of our biological clocks, which follow natural cycles and control the waking/sleeping cycle; oscillatory dynamics are also a fundamental property of neural networks. For example, the central pattern generators enable us to walk without thinking about it, by means of a simple command from the brain, which triggers the working of an oscillatory circuitry in the spinal cord which generates alternating muscular contractions. Implicit time is more generally that which synchronizes our actions with the outside world. It in particular enables us to predict the arrival of a ball or avoid a car. This anticipation is automatic, inherent in the neural dynamics and predicts the future to allow synchronization with the present.

Conscious time, or "explicit time" is for its part far more remote and abstract from physical reality: it is not isomorphic with physical time. Conscious time is first of all the subjectivity of the perception of duration: ten minutes spent in a traffic jam feels like an hour, while ten minutes spent with a loved one feels like a second. Conscious time is also the sequencing



of events: we reconstruct a history on the basis of the causality of events, their logic, their continuity and internal semantics.
The metaphor of passing time illustrates the phenomenological continuity of the perceptual experience of time, which originates in the integrating neural processes. The psychological time engine and the very conscious awareness that a (physical) time engine can exist, are anchored in the working of the brain: psychological time is thus a matter of consciousness.

ÉK: Do you believe that the existence of a psychology of time is enough to prove that of a psychological time? Or would you agree that what we call psychological time is simply a manifestation of our relationship to physical time, a relationship which is replete with psychological factors?

VvW: Based on the dichotomy between implicit time and explicit time in cognitive neuroscience, I believe that one can legitimately consider (physical) time as being an intrinsic dimension of the working of the brain. Various neural, perceptual, motor, cognitive and even attentional phenomena are characterized by different time scales (figure). Thus neuroimaging techniques give us descriptions of the cerebral dynamics to within the millisecond and enable us to infer the evolution over time of the neural processes responsible for the main cognitive functions. However, the (psychological) time to which we have conscious access is, for its part, a history that is carefully reconstructed by the brain and for which the formulation rules are not yet very clear. For example, we represent events along a mental time line with, in our culture, the past to the left and the future to the right (other cultures represent the past behind and the future in front). The mental representations of time do not therefore correspond to physical time; they are the product of neural computations and the cognitive architecture of the system. What we call psychological time is in fact multipartite: it incorporates the perception of duration, of rhythm, of order and causality, of anticipation, or even of quantity. Thus, in the definition of the "time" concept, the main difficulty is to overcome the deep-seated biases of our cognitive heritage.

> Étienne Klein
Saclay Institute of Matter and Radiation (Iramis)
Physical Sciences Division
CEA Saclay Centre

> Virginie van Wassenhove
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

IV.
MENTAL HEALTH AND VULNERABILITY

The articles of this chapter are not available

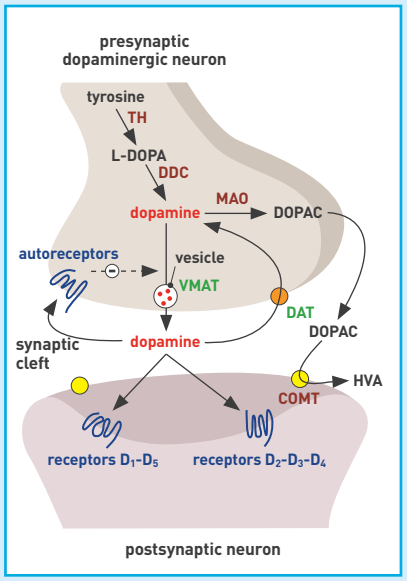
Dopamine

Dopamine is one of the **catecholamines** and is a **neurotransmitter**, a molecule responsible for transmitting information from one **neuron** to another. The dopaminergic neurons which manufacture it are located in the substantia nigra and in the ventral tegmental area, both situated in the midbrain (upper part of the brainstem), and project themselves towards numerous areas of the brain along multiple pathways, including the mesocortical[1], the mesolimbic[2] and the nigrostriatal[3] pathways.

Dopamine is synthesized from **tyrosine amino acid** through the action of two **enzymes**: Tyrosine Hydroxylase (TH), which controls the production of L-DOPA, then DOPA-DeCarboxylase (DDC), which transforms it into dopamine. After synthesis in the pre**synaptic** terminations of the neurons, dopamine is loaded into vesicles by a VMAT transporter (Vesicular MonoAmine Transporter). On the arrival of an action potential (neuronal signal), they release their content into the synaptic cleft (between the neuron releasing the dopamine and that which captures it) by exocytosis[4]. Only some of the extracellular dopamine binds with receptors on the surface of the postsynaptic neuron: the signal is thus transmitted. About 80% of the extracellular dopamine is recaptured by the dopaminergic neurons, thanks to selective DAT transporters (Dopamine Active Transporter) situated in the presynaptic membrane. These transporters therefore precisely control the dopaminergic concentration, both in time and in space. Dopamine can also bind with receptors, called autoreceptors, positioned in the membrane of the dopaminergic neuron.

Dopamine is degraded in the synaptic cleft by an ectoenzyme (COMT), or inside the presynaptic neurons by **mitochondrial** enzymes (MAO). The first way produces homovanillic acid (HVA) and the second dihydroxyphenylacetic acid (DOPAC). The levels of these two **metabolites** in the cerebrospinal fluid respectively indicate the extracellular dopamine level and dopamine synthesis. Five categories of dopaminergic receptors have been identified, the D_1-type family, comprising subtypes D_1 and D_5, and the D_2-type family, comprising sub-types D_2, D_3 and D_4. In the brain, dopamine is also the precursor of **adrenaline** and **noradrenaline**. It can also be manufactured by the hypothalamus, then acting as a **hormone**.

When the production or circulation of dopamine is altered, the nerve cells have trouble communicating, leading to numerous disorders, including Parkinson's disease. Dopamine is also involved in addiction phenomena: the brain releases it during an experience it considers to be "beneficial" (pleasure, reward, etc.).



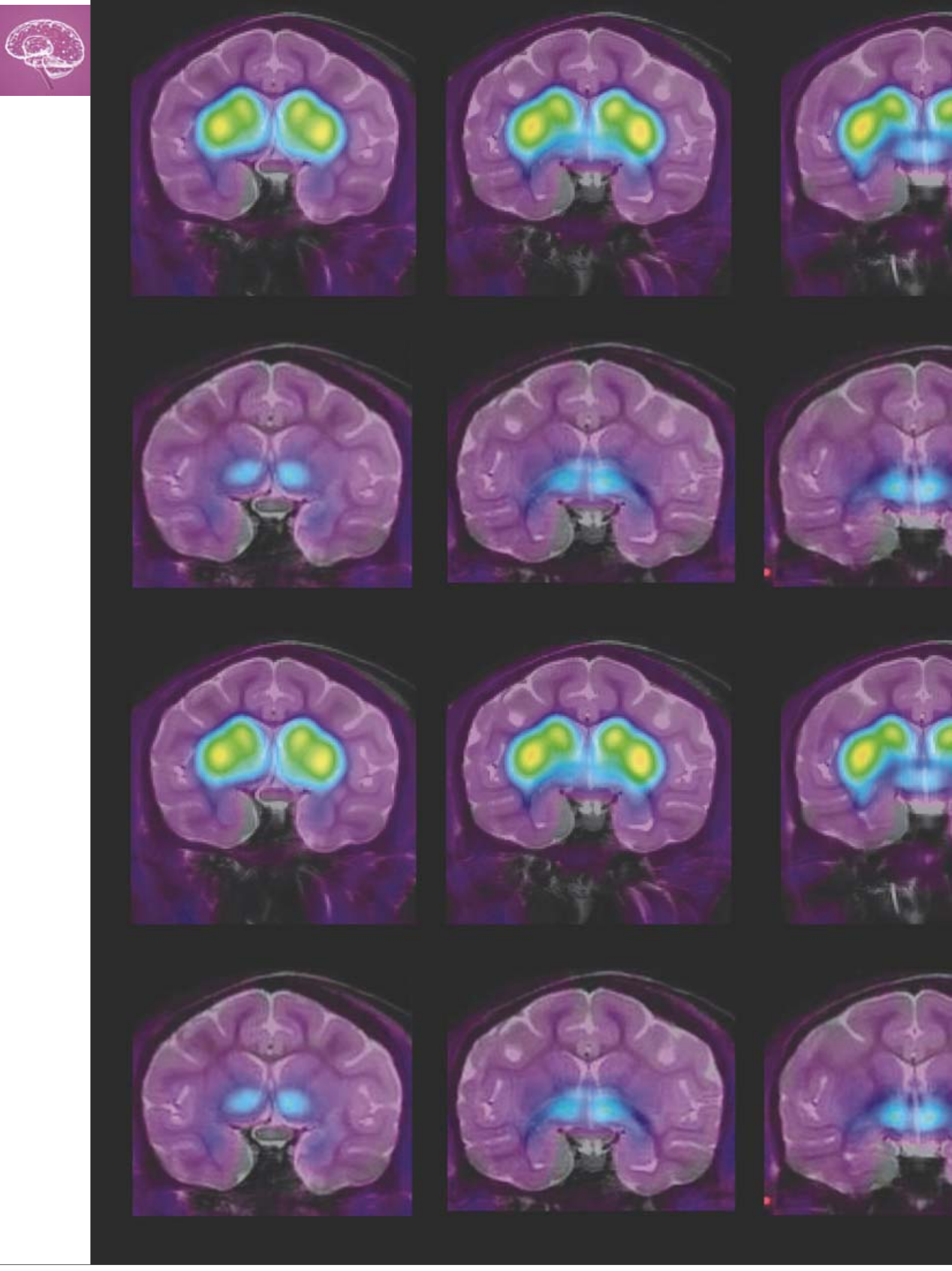
The main steps in the synaptic transmission of dopamine.

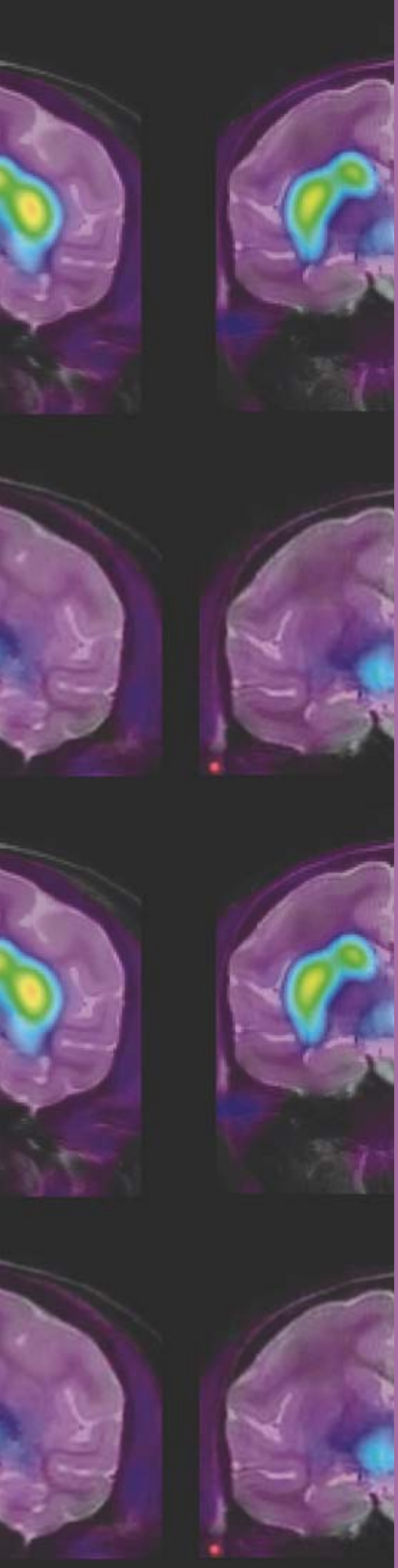
(1) Mesocortical pathway: the targets of the dopaminergic neurons of the ventral tegmental area are the frontal and ventral cortex (anterior cingulate gyrus), the entorhinal cortex and the prefrontal cortex. This neural network plays a role in concentration and the executive functions.

(2) Mesolimbic pathway: the targets of the dopaminergic neurons of the ventral tegmental area are the ventral striatum (nucleus accumbens), the septum, the amygdala and the hippocampus. This neural network takes part in controlling motivational and reward processes.

(3) Nigrostriatal pathway: the target of the dopaminergic neurons of the substantia nigra is the upper part of the striatum (caudate nucleus and putamen). This neural network intervenes in the control of motor functions.

(4) Exocytosis: the vesicles containing dopamine merge with the presynaptic membrane and the dopamine is released into the extracellular medium.





Measurement of the integrity of the presynaptic dopaminergic neuron function using PET on an MPTP primate model of Parkinson's disease (rows 1 and 3, before lesion, rows 2 and 4, after lesion).

CEA/MIRCen

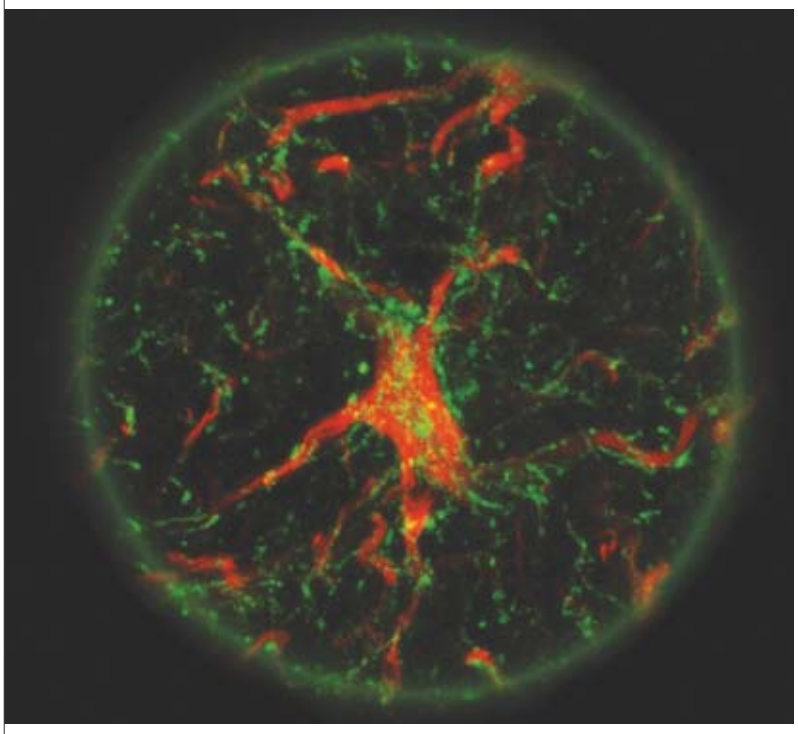
V. NEURODEGENERATIVE DISEASES

Neurodegenerative diseases are a heterogeneous group of pathologies responsible for cognitive and/or motor disorders. For most of them, the diagnosis remains uncertain and as yet there is no curative treatment. Although some may appear in children or young adults, most become apparent in later life. Increased life expectancy is thus indicating a foreseeable rise in the number of patients. Faced with this worrying fact, validating new therapies has become a public health priority.
These diseases are characterized by lesions appearing in different regions of the nervous system through cellular degeneration mechanisms which are still largely unknown. Depending on the case, they affect the central nervous system or the spinal cord exclusively, cause diffuse or localized lesions and result in a wide variety of clinical presentations and syndromes. Some thus manifest themselves as cognitive deficiencies, leading to dementia (Alzheimer's disease), others entail motor problems (amyotrophic lateral sclerosis, Parkinson's disease), while others combine the two (Huntington's disease, Creutzfeldt-Jakob disease).
There are however similarities between the neurodegenerative diseases. This is in particular the case with the accumulation of proteins of abnormal conformation in the nerve tissue, frequently encountered in certain pathologies now known as proteinopathies. Although the process is common, the type of aggregate is specific to each disease. One current hypothesis is that these accumulations lead to the gradual death of the neurons. Proteinopathies would then be the result of a common lesional process. If this were the case, there could be hope for developing a "universal" treatment for all these diseases.
Apart from their clinical and anatomo-pathological diversity, the transmission modes also vary widely depending on the pathology.
A minority of them has a recognized genetic and thus hereditary component, but most occur spontaneously. The search for a diagnostic marker and for a therapeutic approach is therefore not only a major challenge, but also a socio-economic necessity.

> Philippe Hantraye
Institute of Biomedical Imaging (I2BM) – MIRCen
Life Sciences Division
CEA Fontenay-aux-Roses Centre

Understanding **neurodegeneration** mechanisms: the contribution of **modelling**

Animal models of neurodegenerative diseases are being developed in non-human primates. **These are more pertinent than their rodent counterparts and are part of a translational approach aiming to effectively transfer discoveries from laboratory to clinical applications.** Early warning markers of diseases are thus being tested today, before testing/evaluating the efficacy of therapeutic strategies.



CEA/S. Lavisse *et al.*

The astrocytes provide the neurons and synapses with the substances they need to function. They are closely interwoven within the brain's tissue. On this PET image, the astrocytes appear in red and the cell bodies of the neurons can be seen thanks to their green nucleus. In most neurodegenerative diseases, activation of the astrocytes accompanies the disappearance of neurons. This is why they are biomarkers of interest for monitoring the evolution of these pathologies.

Even today, despite intense research, the causes of neurodegenerative diseases remain to a large extent unexplained (see box on *The main neurodegenerative diseases: origins and symptoms*, p. 59). Furthermore, the lack of understanding of the underlying physiopathological mechanisms and the absence of any adequate model make it very hard to develop new therapeutic approaches. In this context, modelling one of these pathologies in animals means attempting to partially or fully reproduce the symptoms and/or lesions characteristic of the disease.

Parallel evolution

At the same time, this approach also allows experimental testing of a number of neuropathological hypotheses formulated on the basis of clinical observations and/or *post mortem* observations on autopsied patient brains. The validity and pertinence of the animal models thus created will then depend on the one hand on the accuracy of the observations and the etiological hypotheses resulting from clinical research and, on the other, on the ability to mimic/reproduce in animals the lesions observed in the patients. The animal models are evolving in parallel with our improved understanding of the diseases, becoming gradually more pertinent – in other words more accurately reproducing the hypothesized cellular/molecular mechanisms – and thus more predictive.

C. Dupont/CEA

Brain slices marked with different specific indicators. Their thickness is 40 micrometres. This marking allows precise characterization of the cellular properties of each of the brain's structures and is essential in developing treatments for neurodegenerative diseases.

The main neurodegenerative diseases: origins and symptoms

Alzheimer's disease, named after the German psychiatrist and neurologist Alois Alzheimer, who described it in 1906, is the primary cause of neurological handicap. It is characterized by the progressive and irreversible loss of different **cognitive** functions, in particular memory. It affects more than 860,000 people in France. The first symptom is often a loss of recent memories (short-term memory), while older memories remain relatively intact. The disease entails initial lesions of the temporal lobe and in particular the hippocampus – a region that is important for memory – and progresses to the **cortical** level, gradually affecting other cortical areas (parietal, frontal **cortex**, cingulate **gyrus**) and sub-cortical areas (nucleus basalis of Meynert, septum, amygdala). The most extensive **neuronal** damage concerns the **cholinergic neurons** and is accompanied by the appearance of two categories of lesions typical of the pathology: **amyloid plaques** and **neurofibrillary tangles**, made of specific protein aggregates.

The second cause of neurological disorders in elderly subjects, **Parkinson's disease**, named after the English physician James Parkinson, who accurately described it in 1817, affects about 150,000 people in France. From the clinical viewpoint, the pathology is characterized by a trio of motor symptoms, including muscular hypertonia (lead-pipe rigidity), reduced and slower movements (akinesia, bradykinesia) and, in about 60 to 65% of the patients, shaking which occurs when the patient is at rest – in the absence of movement. Apart from these motor signs, the illness is often accompanied by depression and more rarely by cognitive disorders, or even dementia in the most elderly subjects. From the physiopathological viewpoint, the most severe damage concerns the dopaminergic system, in particular the dopaminergic neurons present in two sub-regions of the ventral midbrain: the substantia nigra pars compacta and the ventral tegmental area (see box on *Dopamine*, p. 52). Dopaminergic damage is associated with damage to other **neurotransmission** systems (**serotonergic**, **noradrenergic**, cholinergic, **glutamatergic**, etc.). However, in very rare cases (less than 5%) appearing before the age of 50, the form of the illness is hereditary in nature. Predisposition **genes** have been identified.

Huntington's disease, named after the American physician George Huntington, who described it for the first time in 1872, is a **genetic** disease linked to the **mutation** of a gene **coding** for a protein called huntingtin, whose normal function remains to a large extent unknown. Once mutated, the huntingtin protein is responsible for severe neuronal losses in areas of the brain involved in motor or **higher function** control. This pathology affects about 6,000 declared patients and 12,000 carriers of the gene provisionally free of symptoms. In clinical terms it is characterized by abnormal involuntary movements (dyskinesia, choreic movements), severe cognitive disorder associated with planning troubles (frontal syndrome) and numerous psychiatric signs (anxiety, depression, aggression, etc.). From the physiopathological standpoint, the initial cell damage is mainly noted in the structures situated under the cerebral cortex, called the basal ganglia. It then spreads to different cortical areas, especially the frontal cortex, making this pathology a sub-cortical type of dementia.

For example, the first animal model for Parkinson's disease, only reproduced catatonia, that is hypoactivity and slowness of movement initially observed in patients by the English physician James Parkinson in 1817. It then evolved towards electrolytic lesion(1) then neurotoxic lesion (6-hydroxydopamine)(2) models more or less specifically reproducing the selective striatal **dopamine** deficit. This deficit, identified in 1998 by the biochemist Oleh Hornykiewicz and the physician Herbert Ehringer, both Austrian, stems from the gradual disappearance of dopaminergic **neurons** from the ventral midbrain (see box on *Dopamine*, p. 52).

The animal model thus represents a key step in understanding the molecular and cellular mechanisms underlying the neurodegenerative diseases. Once characterized and provided that it is pertinent, it is also a means of testing the selectivity and specificity of **(bio)markers**. Finally and above all, it is irreplaceable for experimentally testing and validating innovative therapeutic strategies, in a controlled way.

A translational approach

CEA Institute of Biomedical Imaging (CEA/I2BM) is adopting a translational research approach aiming to propose, characterize and measure the therapeutic efficacy of new treatments, first in animals then in humans. This approach involves several stages. In the case of the neurodegenerative diseases, this means: improved understanding of the molecular and cellular mechanisms of degeneration; validation of biomarkers; development and *in vitro* and then *in vivo* evaluation of new therapies in animal models and, finally, the "translation" of these concepts to clinical applications. This process is proving to be lengthy and costly. The teams at I2BM had to overcome different types of problems, such as the absence of pertinent animal models for Parkinson's, Huntington's and Alzheimer's disease, or the lack of major infrastructures able to host the complex experimental protocols which demand Biological Safety Level-2 or -3(3) for small and large animals. They also had to deal with the implementation of new non-invasive imaging equipment and processes usable for the **pre-clinical** and clinical stages and, finally, the effective interaction with clinician colleagues within long-term joint pre-clinical and clinical teams (see box on *The NeurATRIS innovation infrastructure*, p. 62).

(1) Electrolytic lesion: this is done by means of an electrode placed in the brain, which sends a weak electrical current locally destroying the neurons.

(2) Neurotoxic lesion: a neurotoxic substance is injected to destroy the neurons. 6-hydroxydopamine neurotoxin selectively kills the dopaminergic neurons.

(3) Biological Safety Levels (BSLs): the four-level containment classification 1, 2, 3 and 4 describes the rising level of constraints concerning the layout of the laboratory, the internal systems and good working practices.

Ideal models

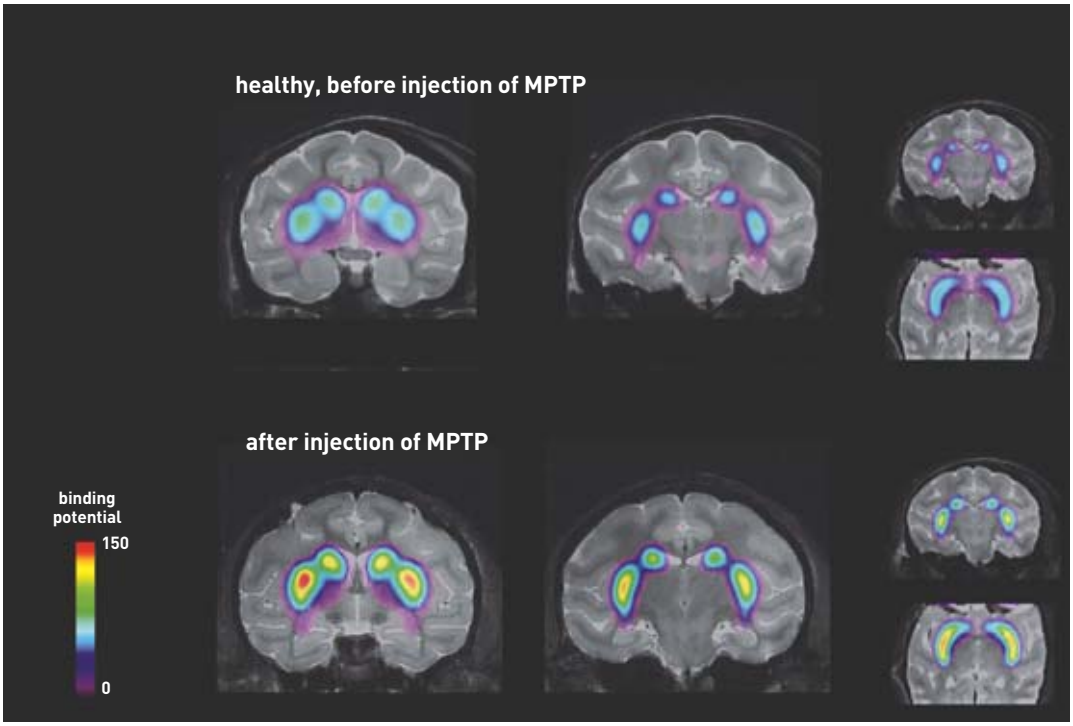
One important aspect of the research carried out concerns the creation and characterization of neurodegenerative disease models in non-human primates. This is because the anatomical organization and functioning of the brains of non-human primates are closer to those of humans than the brains of mice, rats or dogs. During the course of evolution, similar brain structures and working modes have developed in the brains of primates and are not shared by the other mammals. For example, apes and humans give priority to vision as their means of perceiving the world. This was a decisive factor in "conditioning" both the organization of certain **cortical** areas – the visual **cortex** or the frontal control, in particular – and the working of the brain in response to its environment. The executive system responsible for behaviour control is thus organized similarly in all primates. It appears to be significantly different from that of rodents, even if there are some similarities. Non-human primates are therefore ideal biological models for studies in **cognitive** neuroscience and for modelling human pathologies involving disorders of the **higher functions**, such as neurodegenerative diseases. Furthermore, thanks to non-invasive techniques for analysing brain function, which can be used in humans and in non-human primates, it is no longer necessary nor justified to resort to invasive methods. This gives these "large animal" models an additional advantage over the others, in particular for studying the integrated behaviours and higher functions in relation to anatomo-functional deficits visible with cerebral imaging.

World firsts at CEA

The programs developed at CEA in the field of the neurodegenerative diseases thus mainly concern the use of non-human primate models – in addition to the existing **transgenic** rodent models. With non-human primates, it becomes possible to use imaging devices with high translational potential (PET Positron Emission Tomography, MRI Magnetic Resonance Imaging, **MRS Magnetic Resonance Spectroscopy**) and to sample biological fluids (blood, cerebrospinal fluid, etc.) repeatedly. The size of their brains, which is between that of rodents and that of humans, for example allows intra-cerebral injections to be carried out in conditions close to those of the clinic. Thanks to this know-how, the teams were able to achieve several world firsts in cellular and **gene** therapy on the **central** and **peripheral nervous system** (see *Cell and gene therapies: innovative strategies*, p. 63).

A model for Parkinson's

The MPTP primate model for Parkinson's disease is certainly the most closely studied and that with the best predictive capability. It was discovered by chance in 1983 by the American neurologist William Langston (University of Los Angeles), and is obtained by systemic administration of a **protoxin** which, after passing from the blood to the brain, degrades to the MPP+ toxin which specifically attacks the dopaminergic neurons. This model precisely reproduces the motor signs of the disease. It was used as of 1985 in the Frédéric Joliot Hospital Service (CEA/**SHFJ**: **Service Hospitalier Frédéric Joliot**) in an attempt to characterize the properties of the new PET **radioactive tracers**, specific to the



CEA/MIRCen

Distribution of the dopaminergic receptors and of the affinity of the tracer injected for these receptors (sub-family of D_2 receptors) in PET in an MPTP primate model of Parkinson's disease. The PET images (in colour) are superimposed over the morphological images of the same subject acquired in MRI (in grey). The images at the bottom show an increased bonding of the injected tracer to the receptors, following the reduction in endogenous dopamine normally produced by the neurons of the striatum. The MPTP model perfectly reproduces the neuronal degeneration observed in PET imaging in Parkinson's disease.

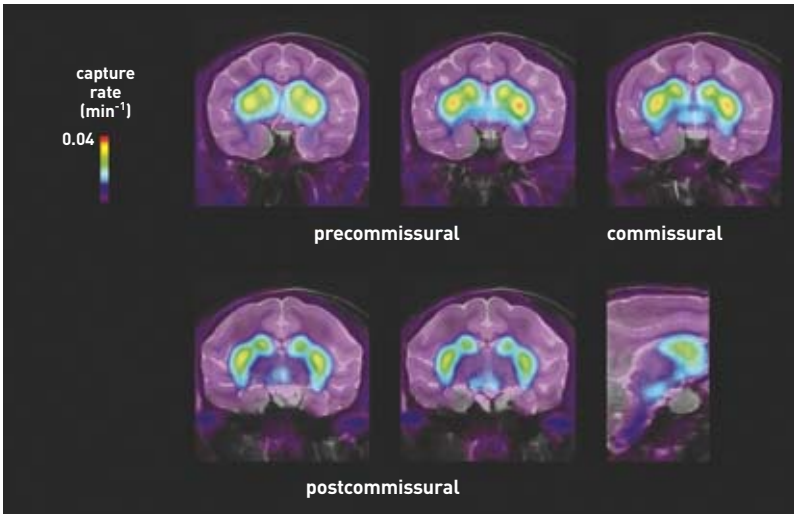
dopaminergic system. When marked by an **isotope** emitting **positrons**, such as fluorine 18 or bromine 76, this type of tracer has the advantage of binding to the dopaminergic receptors and sometimes even to particular sub-types of these receptors. It is then possible to quantify them externally and non-invasively. On the strict condition that their properties of *in vivo* interaction with their protein targets have been beforehand verified, these radioactive tracers can for example be used to characterize – or even quantify – the functional state of a **neurochemical** system in a normal or pathological living subject. This method is so sensitive, that it can detect significant biochemical deficits in non-symptomatic subjects, not yet presenting any motor nor cognitive signs.

This is the case with ^{18}F-Fluoro-L-DOPA, an analogue of L-DOPA – the natural precursor of dopamine, the **neurotransmitter** affected in Parkinson's disease. After injection into the bloodstream, this marker passes through the **blood-brain barrier**, reaches the brain and binds to the DOPA-DeCarboxylase (DDC), a key **enzyme** in the biosynthesis of dopamine (see box on *Dopamine*, p. 52). As the binding of the tracer is proportional to the quantity and/or **catalytic** activity of the enzyme, it provides information about the local density of dopaminergic fibres and/or their intrinsic activity level. The specific bond of this tracer is thus significantly reduced in the MPTP primate (when compared with a normal animal), proportionately to the level of degeneration of the dopaminergic neurons.

Towards improved markers

The highly predictive nature of the MPTP model certainly explains its frequent use in imaging: measurement with **localized proton NMR spectroscopy**, evaluation of the intrinsic sensitivity of other potential markers of the dopaminergic system or markers of neuroinflammation (see below). For Parkinson's disease, the researchers were thus able to evaluate the interest and selectivity of PET markers specific to various proteinic components of the dopaminergic **synapse**, located both on the presynaptic side (recapture system, vesicular dopamine transporters, enzymes involved in the biosynthesis of the neurotransmitter) and postsynaptic side (dopaminergic receptors of different sub-types D_1, D_2...). Most of these markers have been validated for pre-clinical and clinical use and are also utilized in **phase I-II therapeutic tests** for example concerning cell grafts or gene therapy.

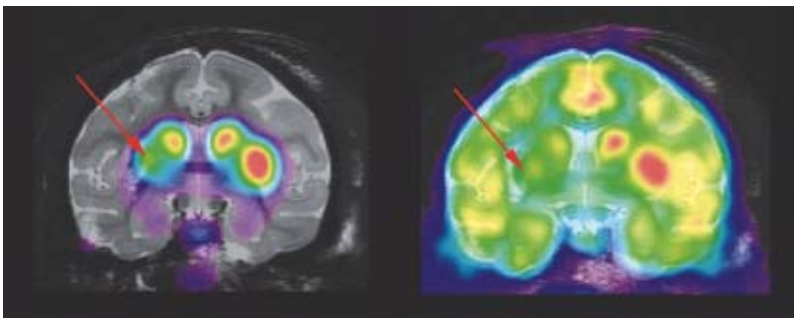
Clinical research has hitherto focused on developing symptomatic treatments, *i.e.* aiming to reduce the severity of the clinical signs. Efforts today are concentrating on identifying molecules or strategies capable of slowing down (disease-modifiers) or blocking (neuroprotectors) the evolution of the disease itself, or even of reinforcing/exacerbating the natural recuperation/compensation mechanisms of the neurons affected. In this new context, one major difficulty is to be able to detect – and if possible quantify – a functional deficit as of the prodromic phase (preceding the appearance of the symptoms) of the disease. Detecting the pathology at this very early stage, while neuronal losses are still relatively limited,



CEA/MIRCen

Measurement of the integrity of the function of the presynaptic dopaminergic neurons with PET (injected tracer capture rate in min^{-1}) in a healthy subject (before injection of MPTP). The PET images (in colour) are superimposed over the MRI morphological images (in grey). The redder the area, the higher the binding of the injected tracer. Conversely, in the areas in purple, the injected tracer is weakly bound because the dopaminergic neurons are poorly represented.

would open an unprecedented therapeutic window. Current research is thus looking at markers of neuroinflammation, a **glial cell** activation phenomenon closely linked to neuronal suffering and which, in most neurodegenerative pathologies, precedes actual neuron disappearance. For about fifteen years, markers belonging to several chemical families have been synthesized and tested at the SHFJ and at CEA/MIRCen in various animal models. To do this, the neuroinflammation mechanism first of all had to be reproduced in an animal model and its temporal evolution characterized, as activation of the glial cells is not stable over time. Here again, specific toxins were utilized to induce a local neuronal loss accompanied by immediate activation of the **microglial cells** and more progressive activation of the neighbouring **astrocytes**. Once characterized from the histopathological viewpoint, this animal model could be used to validate the PET markers potentially selective of the microglial and/or astrocyte cells activated. A specific neuroinflammation tracer was therefore selected at the pre-clinical stage and its absolute quantification developed and validated. It is currently used in three series of clinical trials being performed at the SHFJ, for three neurodegenerative pathologies (Alzheimer's, Parkinson's and Huntington's disease).



CEA/MIRCen

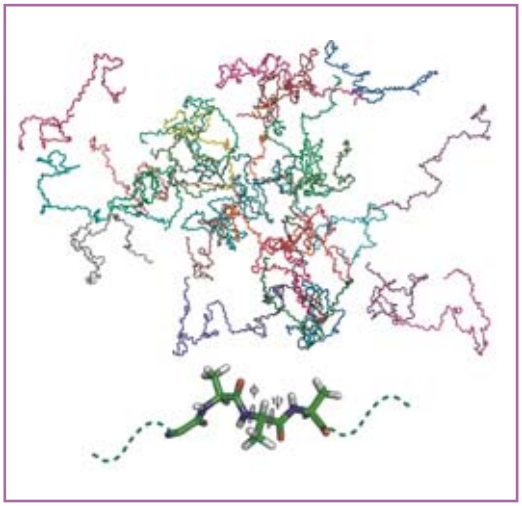
Model of postsynaptic neuron degeneration by injection of quinolinate, a specific toxin capable of inducing localized neuronal loss. On the left, the images represent the density of the dopaminergic receptors [receptors situated on the postsynaptic neurons (in colour)], and by superimposing PET on MRI, they show a loss of neurons in the area where the quinolinate was injected. On the right, the images of regional glucose metabolism, after superimposing PET on MRI, reveal a hypometabolism correlated with the neuronal loss visible on the left-hand image.

Reproducing protein aggregation

At the same time, new PET markers, potentially specific to protein aggregates of Alzheimer's disease, have been discovered. Numerous studies performed with ^{11}C-PIB(4), considered to be selective of the **amyloid plaque**, have revealed its potential but also certain limitations. Its selectivity for the amyloid plaque is in fact relative, because it also exhibits a form of *in vivo* affinity for other highly structured protein aggregates such as **neurofibrillary tangles** or **myelin**. It would therefore appear necessary to develop primate models reproducing all or some of the cellular and molecular lesions characteristic of each of these proteinopathies, in order to characterize the specificity and above all the selectivity of the new markers of protein aggregation which are appearing in the literature.

The MIRCen laboratory is currently studying the effects of intra-cerebral injections of viral vectors (see box on *Viral vectors for gene transfer*, p. 65), which induce a local over-expression of **mutated** proteins and thus reproduce the phenomenon of aggregation specific to Parkinson's disease (mutated alpha-synuclein protein), Huntington's disease (mutated huntingtin protein) or Alzheimer's disease (mutated tau protein). Apart from characterization of radioactive tracers, these models are also used to attempt to elucidate the molecular mechanisms behind the formation of these protein aggregates and study the functional consequences (behavioural, biochemical, electrophysiological, etc.) of their gradual accumulation.

> **Romina Aron Badin** and **Philippe Hantraye**
Institute of Biomedical Imaging (I2BM) – MIRCen
Life Sciences Division
CEA Fontenay-aux-Roses Centre



IBS/Martin Blackledge

The tau protein plays a crucial role in Alzheimer's disease. The protein forms aggregates which lead to neurofibrillary tangle. This is why it is the subject of numerous studies, in particular at the Institute of Structural Biology (CEA/IBS), in order to understand why and how it becomes pathogenic. The highly flexible tau protein adopts numerous conformations, about twenty of which are represented here.

(4) PIB: Pittsburgh Compound-B, name given by the laboratory that developed it.

The NeurATRIS innovation infrastructure

The modern translational approach to research, involving a continuity of research activities from the identification of therapeutic targets to the proof of clinical concepts, is crucial to the implementation of new methods of prevention, diagnosis and treatment, capable of alleviating the economic and social burden of the neurodegenerative diseases. The combination of fundamental research and clinical application does however entail a number of difficulties, in particular with a relatively slow and costly development process accompanied by a high rate of elimination of candidate drugs. Among the main obstacles identified are the lack of animal models to validate the targets, poor predictability of the therapeutic efficacy in humans and a severe shortage of non-invasive **pre-clinical** and **clinical** tools and methods for measuring therapeutic efficacy.

Owing to the multidisciplinary nature of the translational research programs applied to brain disorders and the need to integrate the various steps of complex processes, it is essential to promote the creation of innovation platforms and open them up to academic, clinician and industrial researchers, in order to stimulate the discovery of new therapeutic strategies. Financed by the Investing in the Future Program (**PIA**: **Programme d'investissements d'avenir**), NeurATRIS is designed to reinforce the translational research by providing academic and industrial researchers with a wide range of know-how in neurology and the neuroscience (cell and **gene** therapies, drugs, imaging, etc.). NeurATRIS gathers several research centres(1), coordinated by CEA, with a "one-stop shop" at **MIRCen** which handles the customer's requests and monitors the projects step by step, calling on all the structures concerned. This innovation infrastructure is thus capable of proposing services or performing any clinical or pre-clinical cell or gene therapy trial. This service is unique in Europe.

(1) The three imaging platforms at CEA (MIRCen, **NeuroSpin**, **SHFJ**), the Translational neuroscience university hospital institute at the Pitié Salpêtrière (**Institut hospitalo-universitaire (IHU) de neurosciences translationnelles de la Pitié Salpêtrière**), the BIRD consortium (**I-Stem**, **AIM**, **Généthon**, **ATGC**), Henri Mondor Hospital in Créteil and Bicêtre Hospital in Kremlin-Bicêtre.

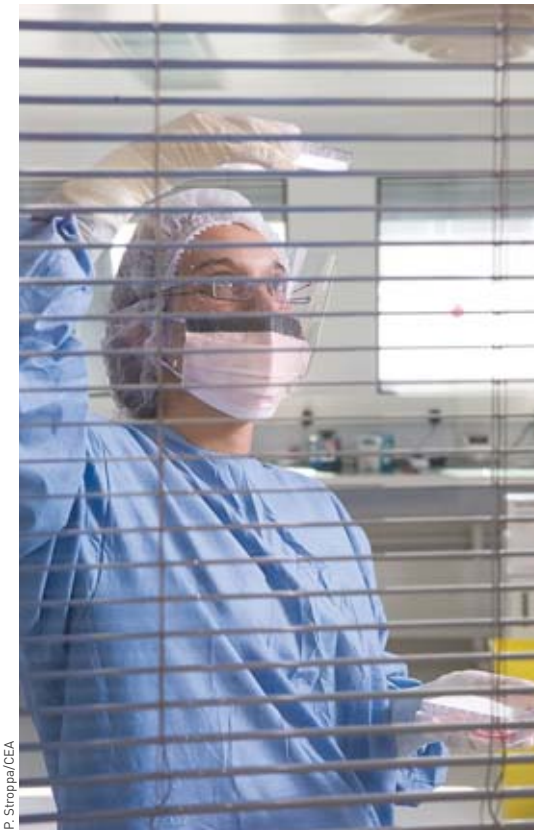
> **Simone Mergui**
Institute of Biomedical Imaging (I2BM)
Life Sciences Division
CEA Fontenay-aux-Roses Centre

Cell and **gene therapies**: innovative strategies

To go beyond simply treating the symptoms of neurodegenerative diseases, we must attack their fundamental mechanisms. This is being attempted by new approaches based on gene or cell therapies and which aim to compensate for the lost functions or protect the neurons, thus blocking the progression of the illness. Although still far from routine clinical applications, they have today reached the stage of pre-clinical trials on animal models, or even phase I or II clinical protocols.

Caring for patients afflicted by neurodegenerative diseases today mainly consists in treating the symptoms (see box on *The main neurodegenerative diseases: origins and symptoms*, p. 59). This is for example the case of the administration of L-DOPA to compensate for the lack of **dopamine** in patients affected by Parkinson's disease (see the boxes on *Dopamine*, p. 52 and *Parkinson's disease and therapies*, p. 66). This situation is the result of our still insufficient understanding of the underlying mechanisms of neurodegeneration. All these approaches use small molecules passing through the **blood-brain barrier** to reduce the scale and/or the severity of the symptoms presented by the patients. Alternative, non-pharmacological methods – cell and gene therapies – are thus being studied, with the aim of restoring and/or repairing cellular assemblies, or stopping or slowing down the progression of the disease.

Cell therapies undertake to rebuild the damaged components of the **neuronal** circuits (grafts of **glial cells** or neurons) in order to restore a normal function, or to introduce cells producing neuroprotective and/or **neurotrophic factors** to preserve a function or facilitate functional recovery. For its part, gene therapy comprises several approaches. These approaches are designed to "protect" the **nervous system** by blocking a **mutated gene** or, on the contrary, to encourage the expression of therapeutic genes – those of trophic factors of the nervous system – or those producing the missing **neuromediators**. Most of these strategies have been or are still the subject of **pre-clinical studies** in different CEA laboratories and, for some of them, of **phase I-II clinical studies** in partnership with **AP-HP** (*Assistance publique-Hôpitaux de Paris*), in particular the neurosurgery and neurology departments of Henri Mondor Hospital in Créteil.



P. Stroppa/CEA

Cell culture laboratory with Biological Safety Level-3 at MIRCen, for the production of the viral vectors used in gene therapy for pre-clinical trials.

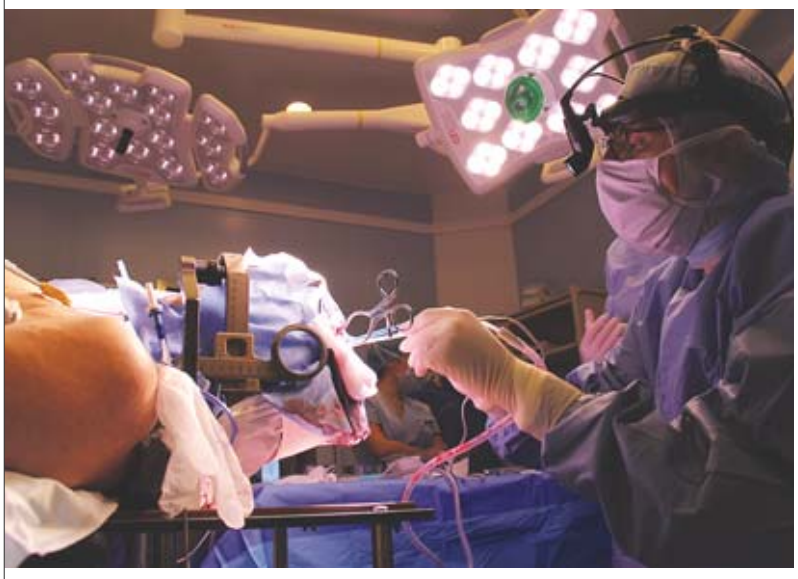
Neuronal grafts

Cell therapy for diseases of the **central nervous system** is still at an experimental stage. Current techniques are based on the use of immature but **differentiated** tissues from terminated embryos. Patients suffering from Parkinson's and Huntington's diseases have thus benefited from a neuronal graft of embryo cells, during phase I-II biomedical studies. These cell transplants led to variable recovery from symptoms such as akinesia (rarity and slowness of movements) and rigidity for Parkinson's disease, or a reduction in chorea (involuntary abnormal movements) and a stabilization of **cognitive** deficits for Huntington's disease. Pre-clinical research on non-human primate models of these pathologies had shown a certain therapeutic efficacy. These clinical trials have demonstrated the validity of the concept in humans, proving the capacities of neuronal circuit restoration, revealed by MRI and PET imaging.

The experience acquired during these biomedical studies however revealed problems at several levels of the procedure, from the collection, availability and survival of the cells to be transplanted, to the **immunosuppression** required or even patient selection. It will thus be necessary to go back to working on animal models before initiating further clinical trials. This is the case with European projects such as **NeuroStemCell**, coordinated by the Italian neurobiologist Elena Cattaneo, and **TRANSEURO**, coordinated by the English neurologist Roger Barker. These studies are being financed by the 7th framework program for research and technological development and their main goal is to overcome problems with the collection of dopaminergic cells usable for routine clinical applications.

Using stem cells

These scientific and technical difficulties are combined with the major ethical hurdles associated with the collection of the embryo cells to be transplanted. It is this complicated context, which seriously restricts the development of a routine therapeutic strategy, which stimulated research into pluripotent stem cells, offering a very real alternative to embryo cells. These undifferentiated cells, capable of self-renewal and proliferation in a culture, can evolve into a particular type of cell, provided that they have first undergone an appropriate *in vitro* differentiation procedure. While cell therapy using stem cells is beginning to be used in phase I clinical trials (demonstration of harmlessness) for certain pathologies of the eye, heart or liver, its use for treatment of neurodegenerative diseases is still at the pre-clinical stage. *In vitro* protocols for differentiation of human embryo stem cells into dopaminergic neurons and **GABAergic neurons** have been described for Parkinson's and Huntington's diseases respectively. The therapeutic potential of these cells is currently being evaluated in the corresponding non-human primate models. If successful, these studies will lead to clinical applications, following on from the tests previously performed with embryonic **neuroblasts**.



AP-HP

Surgery on a patient suffering from Parkinson's disease, to install the system injecting the viral vector into the striatum.

Expressing genes of interest *in vivo*

Gene therapy consists in enabling the very cells of the tissue to be treated to express a gene. By direct administration into the brain, this technique offers a long-term and continuous input of the **proteins** of therapeutic interest behind the blood-brain barrier, which is often impenetrable to large molecules. Moreover, the input of therapeutic **transgenes** into a clearly defined area of the brain induces their local expression, which can be a considerable advantage in neurology, because the specificity and anatomic heterogeneity of the brain often rule out the use of systemic treatments. The systems today considered to be the most effective in achieving the expression of genes of interest (*in vivo*) in the nervous tissue, use vectors such as **lentiviruses** (derived from the Human Immunodeficiency Virus HIV), **adenoviruses** (human, canine, equine) and **Adeno-Associated Viruses** (**AAVs**) – see box on *Viral vectors for gene transfer*, p. 65.

The frequently multigenic origin of diseases of the nervous system seriously complicates the development of such strategies. Inhibiting the synthesis of a toxic protein would however seem to be of therapeutic interest for monogenic pathologies such as Huntington's disease (by blocking the production of mutated huntingtin) or **Friedreich's ataxia** (by inhibiting the mutated ataxin). Pre-clinical studies on animal models of Huntington's disease(1) have proven the therapeutic potential of this approach. A gene coding for a small **interfering RNA** directed against the mutated huntingtin was transferred to a rodent, which led to the degradation of this protein. These studies demonstrated the feasibility of the method but revealed the difficulties of its clinical application. The next step, currently under way at CEA/**MIRCen**, consists in a change of scale, by applying the same gene therapy to a non-human primate model of the disease. The brain of the ape shows both anatomical similarities with the human brain and an intermediate size between that of rodents and that of humans.

Protecting neurons...

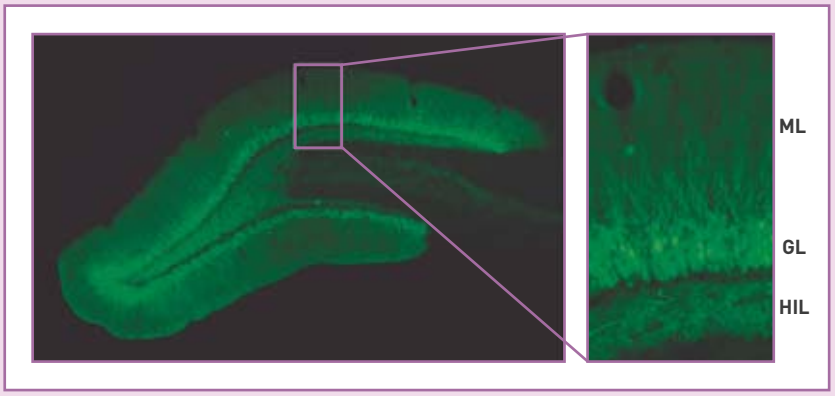
Even though the physiopathology of these diseases remains poorly understood, research into the phenomena of neurodegeneration has identified various mechanisms – **mitochondrial** deficit, faulty detoxification of **free radicals**, activation of specific **proteases**, aggregation of proteins such as mutated alpha-synuclein (Parkinson's disease) or mutated huntingtin – in which the molecular actors could be therapeutic targets (see *Understanding neurodegeneration mechanisms: the contribution of modelling*, p. 58). "Neuroprotection" type gene therapy tools have been designed on this basis. They aim to slow down the degenerative processes by acting at the intra-cellular level. Strategies utilizing trophic factors [GDNF (Glial cell line-Derived Neurotrophic Factor), NRTN (NeuRTuriN), CNTF (Ciliary NeuroTrophic Factor)], the pleiotropic properties of which can enable them to have effects at a distance from their production site,

(1) Transgenic rodents over-expressing mutated huntingtin.

Viral vectors for gene transfer

The principle of gene therapy is to introduce a "drug gene" into a diseased cell, giving it a new function to help it fight the disease. This is in fact a nucleic acid, the genetic information medium, which must penetrate the cell nucleus in order to become active. This nucleic acid, most often a DNA molecule, is incapable of reaching its target on its own. It therefore has to be associated with a vector which will carry it into the cell and its nucleus. The various vectors developed can be divided into two main categories: viruses and... others. This second category comprises purely chemical systems where the negatively charged DNA is complexed and condensed using various molecules such as cationic lipids, polymers or peptides. Although in principle attractive owing to their biosecurity and ease of production, the non-viral vectors currently available do not effectively target the cells in the organism. This is why viruses are used in most cases requiring *in vivo* gene transfer. The specialty of these micro-organisms is to introduce their genetic material into the nucleus of the cells they infect. Several conditions are required for a virus to be able to be transformed into a vector capable of *in vivo* gene transfer.

Firstly, it must effectively infect the target cells, while triggering little or no inflammatory reaction when it is administered. It must then be inactivated in order to eliminate its pathogenic capability. This is done by removing all or some of its genes. The result is a virus capable of injecting its genetic material into a given cell but incapable of replicating in it. This phenomenon is not strictly speaking infection, but *transduction*. Finally, in place of the viral genes, it must be possible to introduce the drug gene and the regulatory sequences which will control its expression.



AAV transfer of the Green Fluorescent Protein (GFP) gene to the neurons of a rat hippocampus. Injection in the vicinity of the dentate gyrus induces the transduction of most of the neurons of this structure, which can then be visualized in green thanks to expression of the transgene (left-hand photo). When highly magnified (right-hand photo), the cell bodies of the neurons in the Granular Layer (GL), their dendrites located in the Molecular Layer (ML) and their axons forming the HILus (HIL) are clearly apparent.

Several types of viral vectors have been successfully tested on the central nervous system. The most promising are currently derived from Adeno-Associated Viruses (AAVs) and lentiviruses. The AAVs are small defective viruses: in the natural state, they require an auxiliary, such as an adenovirus or the herpes virus, in order to replicate. They can infect humans but have no known pathogenic effects. The vectors derived from AAVs therefore have a good safety profile and, when administered *in vivo* into the central or peripheral nervous system, they lead to minimal inflammatory response. Moreover, there are numerous serotypes of AAVs, each with their own tropism, in other words with specific affinity for a given cell-type.

The AAV vector does however exhibit certain limits. On the one hand, the limited size of its genome prevents it from carrying large genes or complex systems for regulating the expression of the transgene. In addition, it does not integrate into the chromatin of the target cell. It cannot therefore be used long term on dividing cells because it is gradually lost by the descendants of the transduced cells. Lentiviral vectors do not exhibit these drawbacks. Their transport capacity is double that of AAVs. In addition, like all members of the retrovirus family to which they belong, their genome integrates into the chromatin of the infected cell. Therefore, when the cells divide, they are transmitted to all the descendent cells.

Finally, when creating a vector, targeting is a major constraint to be considered. It is sometimes possible to act on the vector itself, for example by selecting an AAV serotype specific to the target cells. The other possibility is to use promoter sequences restricting the expression of the transgene to these same cells.

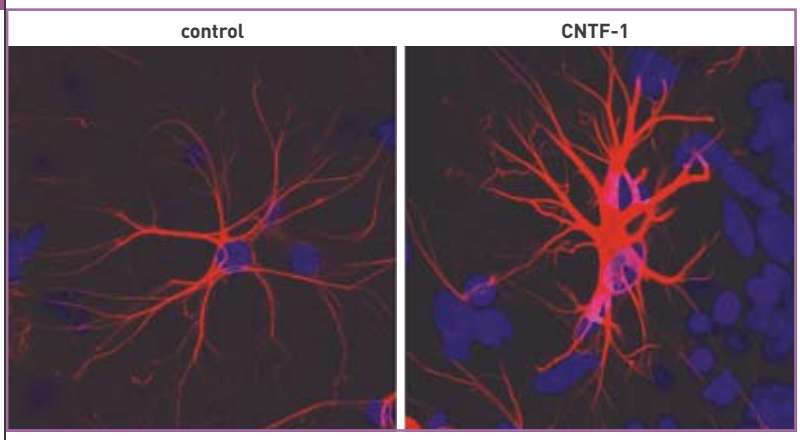
> Alexis Bemelmans
Institute of Biomedical Imaging (I2BM) – MIRCen
Life Sciences Division
CEA Fontenay-aux-Roses Centre

are thus considered to have significant therapeutic potential. Some of them are being investigated in patients suffering from Parkinson's disease in the United States (Ceregene, California).

Similarly, the administration into the striatum of a cytokine (CNTF) has demonstrated therapeutic efficacy in animal models (rodents and non-human primates) of Huntington's disease. It is therefore possible to envisage a neuroprotective strategy for this disease. A pre-clinical trial in non-human primates, using a clinical-quality viral vector, is ongoing at CEA/MIRCen. If it proves to be effective with respect to choreic motor symptoms, this procedure will be applicable to patients during a phase I-II clinical trial conducted at Henri Mondor Hospital.

... or restoring a function

Certain biochemical modifications responsible for the symptoms of Parkinson's disease – such as the low level of dopamine synthesis (see box on *Dopamine*, p. 52) – could be corrected by a gene



CEA/G. Bonvento

Quiescent astrocyte (control) and astrocyte activated by CNTF. The astrocytes respond to pathological situations by becoming reactive. They are activated by a protein, CNTF, the neuroprotective effects of which have been shown. This trophic factor thus offers considerable therapeutic potential for neurodegenerative diseases. Its therapeutic efficacy has been demonstrated in animal models of Huntington's disease thanks to a gene therapy approach consisting in using a lentivirus vector to induce over-expression of the CNTF in the striatum.

therapy strategy aiming at "replacing or restoring" the functions. The technique consisting in locally and continuously inputting dopamine by the expression of the genes involved in its biosynthesis, referred to as the **enzymatic** approach, is already

Parkinson's disease and therapies

Parkinson's disease is caused by the degeneration of the **neurons** producing **dopamine**, a **neurotransmitter** that is essential to controlling the body's movements (see box on *Dopamine*, p. 52). The reference treatment consists in oral administration of L-DOPA, a dopamine precursor drug. A real improvement in motor activity is observed in the first stages of the illness, but severe side-effects appear after a period of time: fluctuating effect of the treatment and involuntary abnormal movements (dyskinesia). This is apparently the result of daily one-off administration of the treatment, which does not allow continuous stimulation of the neurons nor impregnation of all of them. Another therapeutic approach is deep cerebral stimulation by electrodes. This proves to be highly effective on motor fluctuations and indirectly on dyskinesia, but remains invasive and restricting.

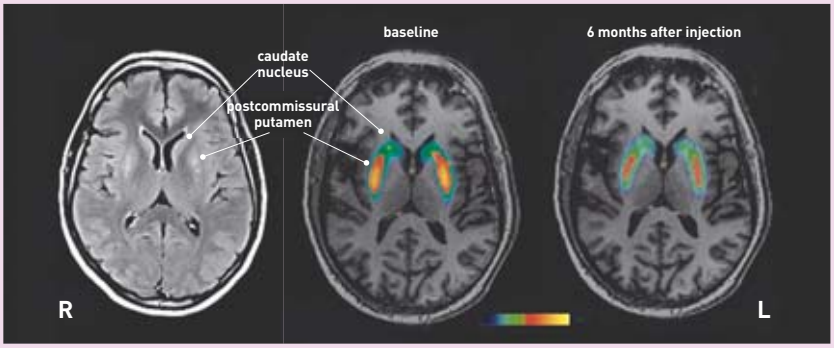
The researchers therefore turned their attention to gene therapy. A **phase I-II clinical trial** was performed by a Franco-British team[1] on 15 patients suffering from an advanced form of Parkinson's disease. The treatment consisted in injecting a **lentiviral** vector into the striatum, expressing the **genes** of three **enzymes** essential to the synthesis of dopamine. The lentiviral vector used was ProSavin®, manufactured by the English company Oxford BioMedica. It is developed from the Equine Infectious Anemia Virus (EIAV). **Pre-clinical studies**, conducted since the early 2000s, had demonstrated the efficacy and harmlessness of this therapeutic approach in rodent and primate models of Parkinson's disease, and thus paved the way for this clinical study.

Thanks to corrective genes, the cells in which the viral vector is injected begin to produce and secrete dopamine. This therapy induces continuous, localized dopaminergic stimulation: the patient regains a good level of motor control, while avoiding the neuropsychological complications linked to the stimulation of other brain areas not affected by the disease. In all the patients in this clinical trial, the motor symptoms of the disease were improved up to 12 months after administration of the treatment. It was also demonstrated that a stronger dose of the lentiviral vector administered resulted in greater therapeutic effects. Now with four years of hindsight, this study demonstrates for the first time in humans the harmlessness and the tolerance of the expression of genes directly introduced by a lentivirus.

This major step forward in gene therapy will open up new therapeutic prospects in diseases of the **nervous system**.

CEA

Pre-clinical trial in an MPTP primate model of Parkinson's disease, aimed at studying the efficacy and harmlessness of the lentiviral vector, ProSavin®. The PET images reveal that the synthesis of dopamine was restored after injection of this drug into the striatum. The benefits of this gene therapy method were thus demonstrated.



CEA

Restoration of the dopaminergic function by gene therapy. The MRI image on the left indicates the anatomical brain areas of a Parkinson's disease patient into which the lentiviral vector was injected. The centre and right images, superimposing MRI (in grey) and PET (in colour), show the reprogramming of the neurons to produce dopamine thanks to the corrective genes. On these images, the more the colour scale tends towards the green, the greater the quantity of dopamine produced by the neurons of the striatum. These images and analyses were made by CEA/MIRCen at the Frédéric Joliot Hospital Service (CEA/SHFJ).

(1) This team comprises AP-HP (*Assistance publique-Hôpitaux de Paris*), Henri Mondor University Hospitals, Inserm, CEA (MIRCen and SHFJ), UPEC (University Paris-Est Créteil Val-de-Marne), Oxford BioMedica and Cambridge University.

under clinical investigation in France (see box on *Parkinson's disease and therapies*, p. 66) and in the United States (Avigene, University of California San Francisco UCSF). This involves directly injecting the genes of interest into the areas lacking in dopamine, in this case the striatum.

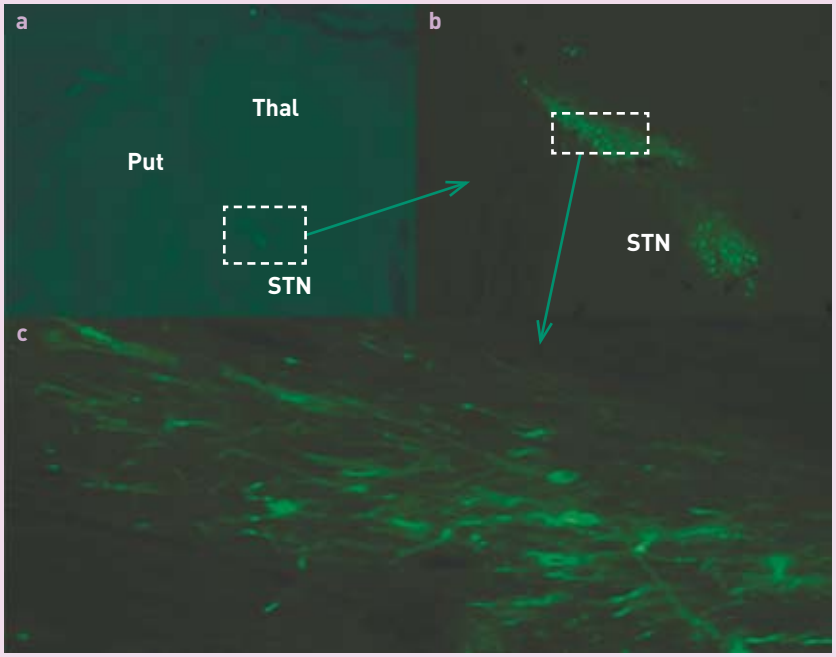
Recent progress in molecular and cellular biology and in vectorology have led to considerable technological advances in the past few years, enabling real clinical applications to be envisaged for the neurological diseases. Proofs of concept on harmlessness and tolerance have already been established in patients. It is now necessary to move onto the subsequent stages of clinical research in order to demonstrate that these innovative gene and cell therapies are real therapeutic alternatives.

> **Philippe Hantraye**[1] and **Stéphane Palfi**[2]
[1]Institute of Biomedical Imaging (I2BM) – MIRCen
Life Sciences Division
CEA Fontenay-aux-Roses Centre

[2]AP-HP – Henri Mondor Hospital
Créteil

Optogenetics for mapping neuronal networks

The working of the neuronal circuits involves a variety of excitatory or inhibitory neurons. In order to better understand the contributions of these cells to the normal or abnormal working of the neuronal networks, it is necessary to study the cause and effect relationships between their activity and the behaviour of the organism. These objectives can only be achieved by artificially, but precisely and reversibly modifying these neuronal activities, while observing the behavioural consequences.
The recently-developed optogenetics method can be used for controlled modulation of neuronal activity on the millisecond scale. It is an association of optics and genetics, combining gene transfer techniques – in this case the ion regulator genes called opsins – with stimulation by light. Opsins are transmembrane(1) proteins, often of bacterial origin, with the property of being able to change conformation in response to light of a specific wavelength. In so doing, they open (or close) channels in the cell's membrane allowing ions to pass. Some opsins admit sodium ions (Na^+), which make the neuron excitable, while others admit chlorine ions (Cl^-), which inhibit it. Through targeted gene transfer, it is therefore possible to make precisely selected neurons "photo-stimulable" and to offer long-term, modular control of their excitation or their inhibition by means of light. Devices called optrodes, combining an optical fibre and a recording electrode, can now be used to photo-stimulate and at the same time record at the same location, with no significant artefacts.
In the space of just seven years, this technique has already been applied to understand the working of neuronal circuits and develop therapeutic approaches in *in vitro* or *in vivo* brain slice models in rodents, for pathologies such as Parkinson's disease, epilepsy, addictions, medullary trauma, schizophrenia, Obsessive Compulsive Disorders (OCDs), consciousness disorders and blindness. More recently, it was used for the first time in non-human primates with the expression of ChR2 (channelrhodopsin 2)(2) channels in the prefrontal cortex and successful neuronal activation by light, thus potentially opening the door to a revolution in our understanding and treatment of numerous neurological and psychiatric illnesses in humans.



Neuronal and axonal expression of a GFP-ChR2 (Green Fluorescent Protein-channelrhodopsin 2) protein after injection of a viral vector into the SubThalamic Nucleus (STN) of a non-human primate. Thal and Put signify thalamus and putamen respectively.

S. Senova *et al.*, "Optical stimulation of the cortico-subthalamic pathway using lentiviral vector with retrograde transport properties for CHR-2-eYFP in non-human primates", *Society For Neuroscience*, New Orleans, 2012

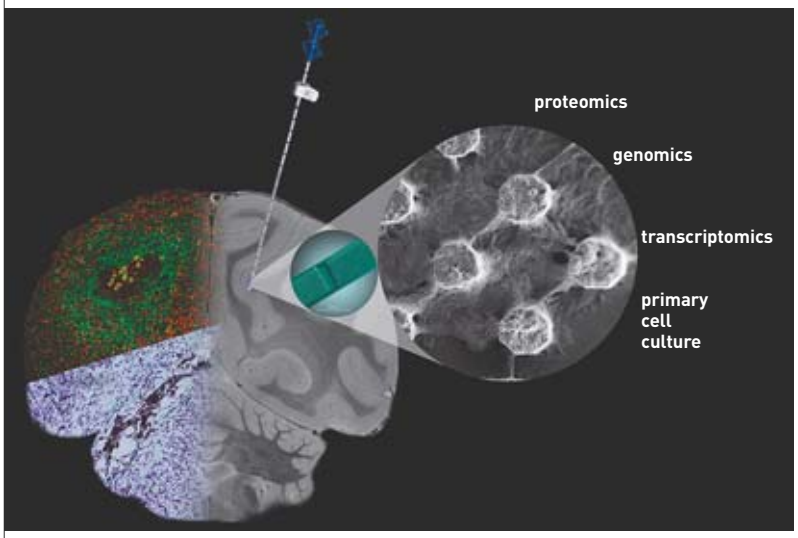
(1) Proteins which are inserted into the cell membrane and which, given their size, pass from one side to the other.

(2) Channelrhodopsin 2 (ChR2), whose gene is taken from unicellular algae, opens in blue light (wavelength 470 nm), allowing Na^+ ions to pass into the neurons, thus making them excitable. Rhodopsins 2 consist of an opsin associated with retinal (one of the three forms of vitamin A).

> **Stéphane Palfi**
AP-HP – Henri Mondor Hospital
Créteil

Implanted **micro-nano-systems**

For a long time, the brain had remained inaccessible for therapy. A quarter of a century ago, neurostimulation made the first steps. **The technologies derived from micro- and nanoelectronics today offer new prospects for cerebral exploration and treatment strategies** (*in situ* stimulation or delivery, assistance, etc.), including for the neurodegenerative diseases.



Clinatec – U1167

Molecular and cellular non-lesional imprint device for exploring the mechanisms of neurological diseases.

The targeted therapies which have revolutionized oncology have had little impact on the pathologies of the nervous system, whether degenerative, traumatic or tumoral. Owing to the inaccessibility of the living brain, the polyomics molecular deciphering of its pathologies has lagged far behind. Moreover, the blood-brain barrier prevents access by numerous drugs to the parenchyma of the brain, and peripheral vectorization strategies have failed to produce the once-hoped-for efficacy. Is the brain therefore beyond the reach of all therapeutic intervention?

A brain-machine interface

Neurostimulation, developed 25 years ago by Professor Alim-Louis Benabid, is an innovative response which opened the door to a promising avenue of research. For example, an electrode implanted in the sub-thalamic nucleus, delivering high-frequency electrical stimulation, neutralizes the motor symptoms of Parkinson's disease. Alongside the drugs, when they begin to lose their efficacy, an implanted technological device is giving convincing therapeutic results, subsequently confirmed in other pathologies such as dystonia, depression or Obsessive Compulsive Disorders (OCDs). This fundamental discovery has opened up the field of micro-nano-systems implanted in the brain. Electronic technologies have now reached a level of miniaturization corresponding to the fundamental dimensions of the living organism, offering unparalleled prospects in terms of multifunctionality and integration.

Brain-machine interfaces have highlighted the crucial role of this miniaturization. The aim here is to address the handicap of a tetraplegic patient by means of a device recording brain activity in the motor area, then decoding it and converting it into a signal controlling an effector, for example an exoskeleton replacing the mobility of the 4 limbs (figure). The electrodes used can be on the surface of the cortex or, on the contrary, implanted closer to the neuronal activity. These devices have been tested in humans, albeit with rudimentary versions, primarily to control the upper limbs. The proof of the clinical concept was provided by the command of robotic arms with a complexity of movement comparable to that of the human arm. Assistive robotics is another field to explore in order to achieve devices that are perfectly tailored to the ergonomics of patients, such as exoskeletons responding to the motor stimuli from tetraplegic subjects. The synergy between the expertise available at CEA's Leti (Laboratory of Electronics and Information Technologies) and List (Laboratory of Integrated Systems and Technologies) makes these developments internationally competitive.

Towards increasingly symbiotic devices

Although deep implants capture neuronal activity with the best resolution, they pose the problem of long-term integration into the brain. The function of the micrometric arrays of electrodes is often neutralized by glial scar formation(1). The availability of more symbiotic electrodes is thus a major technological challenge. Numerous materials derived from nanoelectronics have demonstrated their ability to improve this brain-machine interface, such as carbon nanotubes, nanodiamond or nanowires. The long-term stability of these devices, their persistent functional integration, the absence

(1) Glial scar formation: the glial cells spread and gradually occupy the area damaged by the implantation of the electrodes and form a scar.

of degradation and release of the installed materials do however demand a rigorous analysis of cerebral and systemic biocompatibility.
The resolution of the recordings is also a key issue, for example with recent work implementing new flexible materials with unparalleled deep exploration capacity. **NEMS** (**Nano-Electro-Mechanical Systems**), which have very-high-resolution electrophysiological recording properties, will be technological building blocks vital for current projects to map **cerebral connectomics**. Closed-loop sensory feedback strategies(2) have been developed recently to replicate physiological movement even more closely. However, there is as yet no complete implant integrating electrodes, decoding algorithms and wireless control of the effectors, with the whole system embedded in the patient. To achieve this, it will be necessary to call on the full potential of electronics, which was one of the driving forces behind the creation of the **Clinatec** centre in Grenoble. The technological building blocks put into place for the brain-machine motor interfaces are usable for other locations and other pathologies: application to speech by controlling a voice synthesizer, or even integrated assistance for complex functions such as memory.

Exploring the neurodegenerative diseases

Thanks to their extremely miniaturized 3D integration capacity, the available technologies make it possible to imagine multifunctional implants endowed with high-resolution integrated recording capacities and closed-loop regulated micro-nano-stimulation properties. Localized micro-injection devices employing micro-catheters and micro-pumps, based on **piezoelectric** IT technologies, are already being developed. In the field of optoelectronics, the miniaturization of **light-emitting diodes** is paving the way for embedded optical imaging and stimulation functions enabling neuroprotective light stimuli to be delivered, for example in the **infrared** spectrum.
The implantation of miniaturized devices in contact with the brain is also an unprecedented opportunity to explore the physiopathology of neurodegenerative diseases, thus overcoming a fundamental obstacle to finally envisaging curative therapies for these pathologies. The research carried out at Clinatec has shown that it is possible to collect fresh micro-explants from the brain in contact with the neurostimulation micro-devices and subject them to the most detailed molecular analyses (see illustration p. 68). Second-generation-oriented and non-lesional spatial capture methods – taking a sort of *in situ* brain imprint – have thus been implemented using micro-nano-technologies. This "post-biopsy" medical procedure is an unprecedented method for neuropathological exploration of the brain and one that is crucial for the definition of new targets for the treatment of the neurodegenerative diseases.

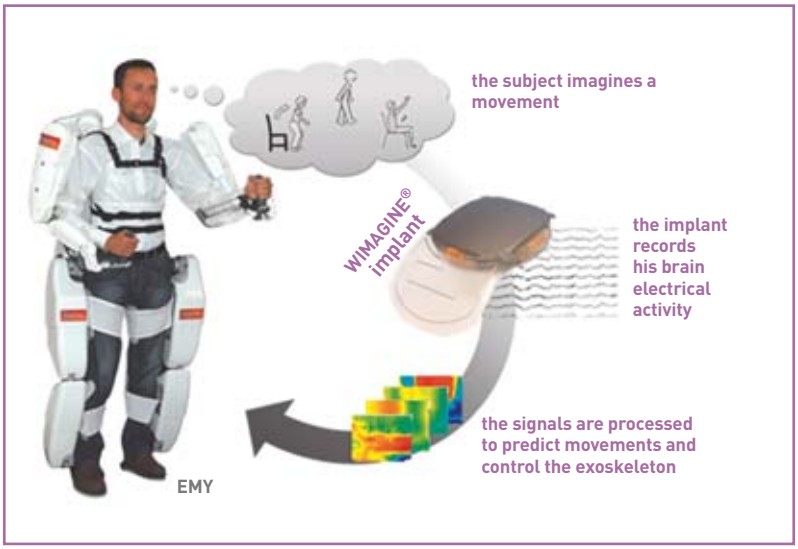


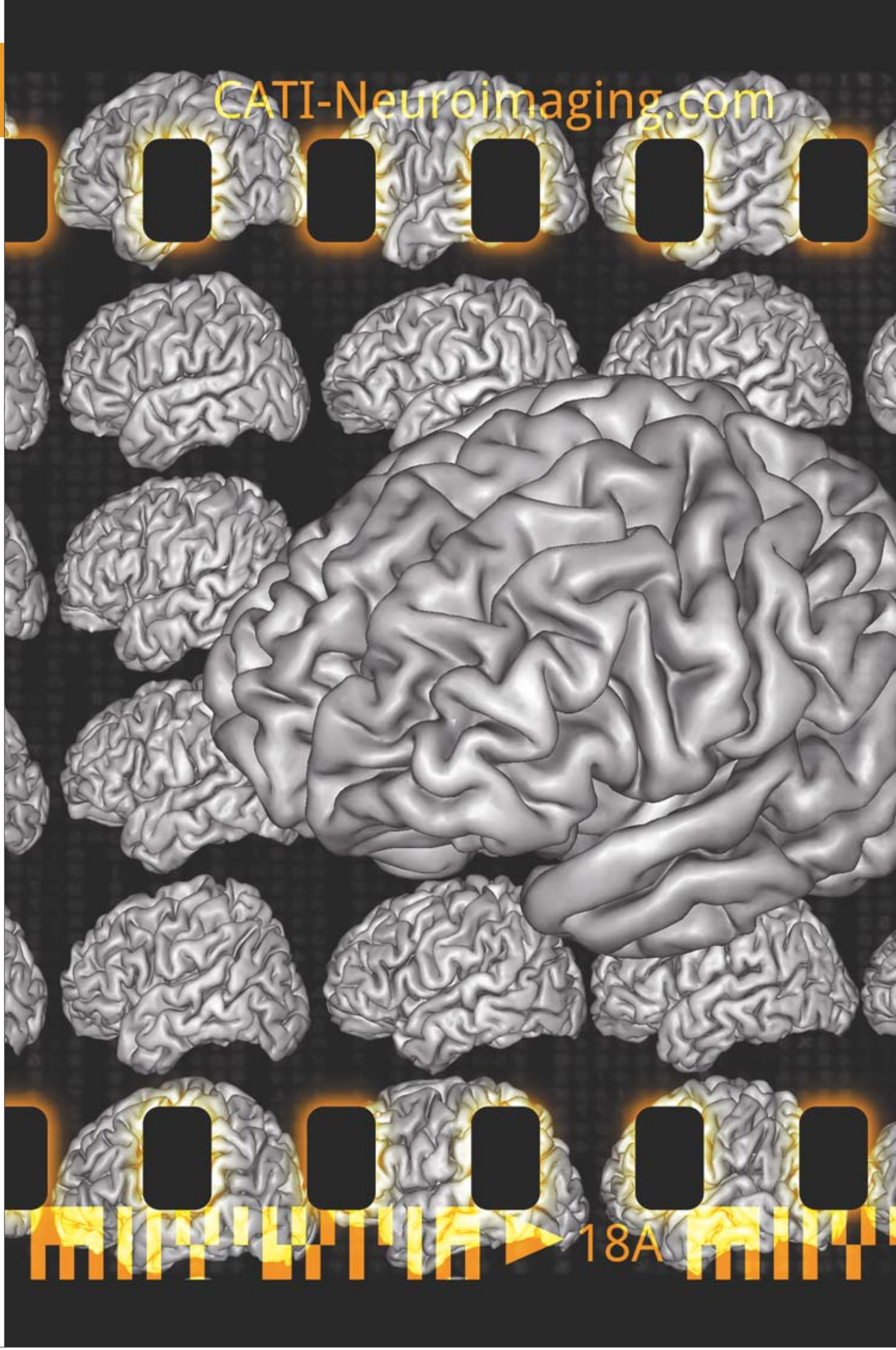
Figure.
Principle of brain-machine interface devices.

A major industrial challenge

To conclude, the micro-nano-technologies derived from IT and electronics offer unprecedented possibilities for intervention in the brain, to treat neurodegenerative diseases. Truly-**theranostic** implanted devices are now being produced, allowing the capture of physical or molecular cerebral activity, the installation of closed-loops and embedded algorithms assisting the complex functions of the brain by effectors that are today motor functions but which might one day be **cognitive**. Localized delivery and stimulation strategies supplement this therapeutic arsenal and, alongside conventional drug-based approaches, constitute a sector in their own right: implanted micro-nano-systems and electronics. A number of social, industrial, regulatory and organizational hurdles still need to be overcome, such as a pertinent analysis of biocompatibility and pre-clinical operability, requiring "large animal" models, the definition of suitable clinical protocols for proof of concept, in dedicated centres ensuring rapid and safe validation in a multidisciplinary techno-biomedical context. The social impact and ethical issues, such as that of human enhancement, must be anticipated. This is also a major industrial challenge.

> **François Berger**
Clinatec – U1167
Leti Institute (Laboratory of Electronics and Information Technologies) – Inserm – Joseph Fourier University – Grenoble CHU
Technological Research Division
CEA Grenoble Centre

(2) According to the theory by the American Jack A. Adams, closed-loop motor learning is the result of the congruence of several processes: a motor memory triggers a movement which produces a sensory feedback, leaving a trace in another memory. The sensory feedback from this movement is compared with the accumulated perceptive trace. Any error between the actual feedback and the expected feedback is detected and the movement is then corrected.


CATI-Neuroimaging.com
18A

Modern data mining today works with bases containing thousands of brains.

J.-F. Mangin, C. Fischer, D. Rivière and G. Operto

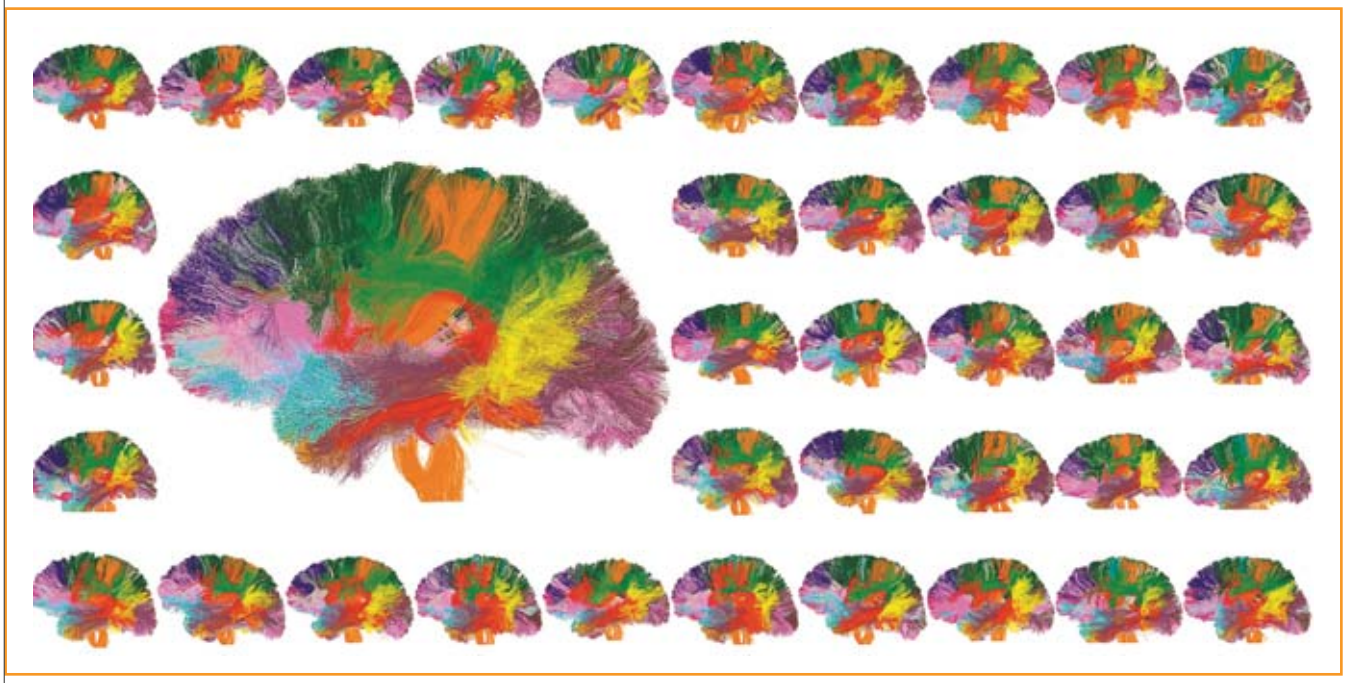
VI. IDENTIFYING BIOMARKERS FOR CEREBRAL DISORDERS

Most brain pathologies and learning disorders nowadays leave the scientific community unable to propose a therapy. The considerable technological progress accomplished in the past decade in the fields of genetics and neuroimaging have however given rise to fresh hope. The international community is today coming together to collaborate on the set-up of gigantic databases on each of the brain's disorders.
The most notable effort is that concerning Alzheimer's disease, a very real burden on our societies. A project gathering together nearly 10,000 subjects has thus been set up, in order to identify the genes associated with the volume of the hippocampus, a structure of the brain involved in memory and which atrophies very early on in Alzheimer's disease. The links between the cerebral phenotype and the human genome are currently the focus of our concerns, but still give rise to much perplexity owing to the underlying complexity and the combinatory explosion that results from the juxtaposition of these two types of data.
Current international efforts are concentrating on detecting biomarkers that only a computer is capable of discovering within these oceans of information. Such biomarkers could make it possible to detect certain weaknesses or the signature of a pathology, before the first clinical signs, thus considerably increasing the chances of success of a preventive therapy. These new biomarkers would simplify therapeutic trials by making it possible to select homogeneous patient groups. Data mining is also liable to be able to stratify the heterogeneous populations associated with certain syndromes into groups of homogeneous patients. The most ambitious project, launched in 2013 by the Human Brain Project, aims to take a fresh look at how we subdivide the spectrum of brain pathologies, by combining all the data from a hundred or so European hospitals.

> Jean-François Mangin
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Information Analysis and Processing Unit
Life Sciences Division
CEA Saclay Centre

Brain research enters the **big data** era

The volume of data is growing and multiplying in the field of brain research, which also appears to be entering the big data era. The increased volume of images generated by neuroimaging, size of monitored patient cohorts and number of analyses conducted are all factors underlying this explosion. To this can be added the regular monitoring of patients, which requires permanent updating of the analyses performed.



D. Duclap, P. Guevara, J.-F. Mangin, D. Le Bihan and C. Poupon

Diffusion imaging is able for the first time to map the variability in the main cerebral communication pathways in thousands of individuals. In the coming years, it is probable that we will discover that some pathologies are the result of anomalies in this connectivity.

The world of research into the brain and its pathologies would clearly seem to be entering the big data era, ushered in with much trumpet-blowing by Google and its competitors. This transition is the result of the explosion in the size and complexity of data and the growth in the longitudinal monitoring of patients, in other words over several years, which demands that the analyses be regularly updated as new data are received. These changes clearly evoke the concept of big data with reference to fields in which data are voluminous, highly diverse (sources, formats, etc.) and multiply rapidly.

An explosion in the volume of data

With regard to the explosion of volumes, the most notable revolution is that of the **DNA sequencers**. They have progressed by a factor of 10,000 in the past decade, *i.e.* 100 times faster than Moore's law, an empirical law which describes the growth in computing power. Such an advance means that, regardless of the scale of the project, it is now possible to envisage mapping of the exome, the **coding** part of the **genome**, or even the whole genome, for thousands of individuals. It should be recalled that a single genome requires 1/3 of a **terabyte** of data storage… This outstripping of Moore's law means that the biologists are now joining forces with large High Performance Computing (HPC) type centres, usually designed for physicists. This is what CEA's Genome Institute at the Life Sciences Division (DSV) is currently doing.

To a lesser extent, neuroimaging is also contributing to the data explosion. A conventional MRI radiological examination is barely more voluminous than a digital photo, even though three-dimensional. However, technologies such as functional MRI or diffusion MRI, now widely used in research, generate far more data. Functional MRI can visualize the functional networks of our brain, *via* variations in oxygen consumption over time, which produces hundreds if not thousands of 3D images for a single examination (one 3D image every 2 seconds). Diffusion MRI is for its part used to map the connectivity of our brain, by imaging the **anisotropy** of the random movements of water within the bundles of fibres. These are nested 3D images: each voxel, the three-dimensional equivalent of a pixel, itself contains a 3D image of the mobility of water in the corresponding cube of the brain. The current trend shows no signs of

slowing down. To speed up and improve the MRI image construction process, the researchers use parallel antennas capable of generating up to a hundred images simultaneously. This technology should become the norm in order to take full advantage of the 11.7-tesla magnet, scheduled for installation in NeuroSpin in 2015, with one aim being to produce "close-ups", and thus increase the spatial resolution of the images…

Increasingly large patient cohorts

Not only the size of the actual examinations needs to be considered, but also the number of subjects involved in today studies. In the field of imaging, it is now common to launch an acquisition campaign on several thousand subjects. This inflation is in particular the result of a new research strategy which consists in acquiring cerebral images at the same time as an extensive map of the genome. In recent years, under the Imagen European Project on addictions, NeuroSpin thus centralized data obtained from 2,000 adolescents in 7 European cities, including the Saclay plateau (Essonne *département*). Given that the number of subjects usually necessary for genetic studies is about several tens of thousands, for sensitivity reasons, it is easy to imagine the rest. Germany for example has just launched a national imaging cohort on 30,000 subjects. Numerous initiatives are aiming to harmonize the acquisition and analysis procedures, to be able to combine the data from several projects (see box on *CATI, a large cohort imaging instrument*).

(1) There are two imagers: one with a magnetic field gradient of 300 mT/m and the other 100 mT/m. This latter was used for the 1,000 subjects. These magnetic field intensity levels are unprecedented for use on humans.
http://www.humanconnectomeproject.org/

Open access to data

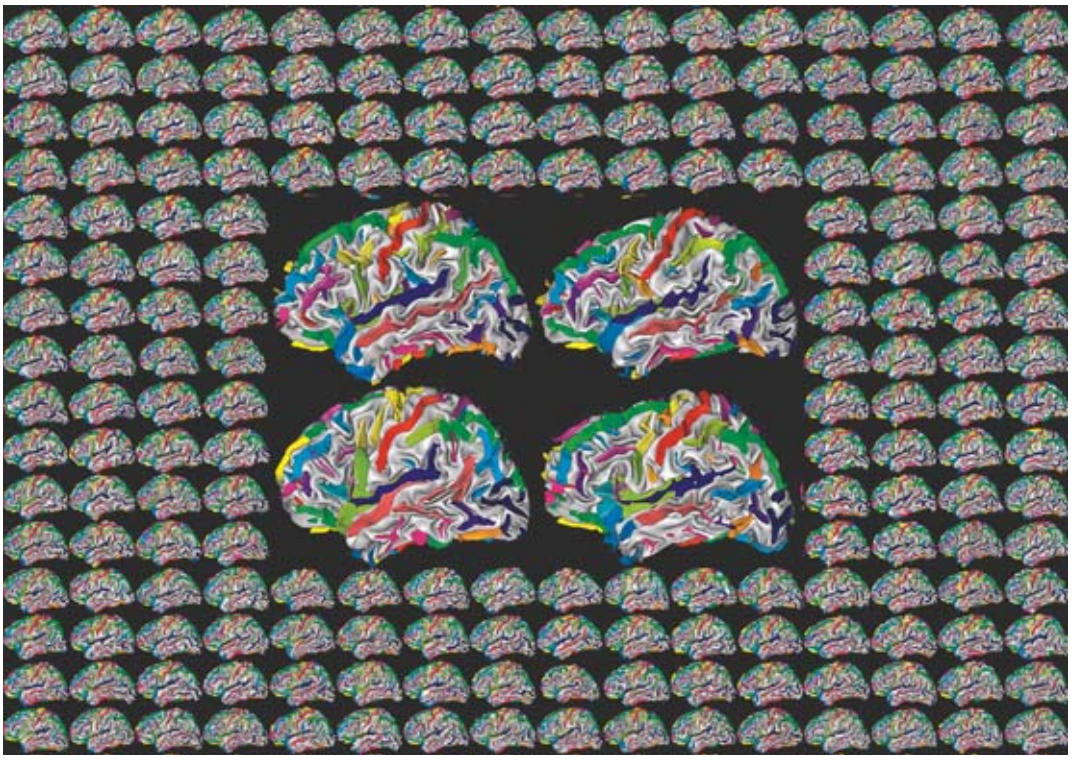
The cost of acquisition projects concerning several thousand individuals rapidly reaches tens of millions of euros. This has resulted in a major change: the agencies which fund these projects are increasingly demanding open access to the data for the international community as a whole. This is the case with the Human Connectome Project, financed by the American National Institutes of Health (NIHs) and giving access to the data acquired from a thousand individuals using a performant imager[(1)] dedicated to mapping the connectivity of the human brain, a field as yet poorly understood. The free access strategy reinforces the impact of the database, thus justifying the cost of its financing. An international organization, the International Neuroinformatics Coordinating Facility (INCF), is supporting this move towards open data by facilitating international cooperation in the field of neuroinformatics.
Under the Human Brain Project, mainly financed by the European Community, the teams at NeuroSpin will also be running a project capable of generating a huge database: about ten subjects will be asked to take part in a decade-long study which aims to perform on them all the functional MRI protocols designed around the world. These subjects will be the cosmonauts of brain research and will take part in exploring it, side by side with the researchers.

A wide diversity of data

The variety of data can be explained by two factors: the complexity of the brain, with its multitude of anatomical structures and functional networks, and the large numbers of acquisition technologies and image processing software. The image analysis tools developed over the past thirty years have also reached such a level of performance that it is now possible to envisage systematically

CATI, a large cohort imaging instrument

The Alzheimer's disease Image Acquisition and Processing Centre (CATI: *Centre d'acquisition et de traitement d'images pour la maladie d'Alzheimer*) was set up by a group of Île-de-France region laboratories with complementary expertise: NeuroSpin, CEA's high field MRI imaging centre, and several research teams specializing in neuroimaging and dementia from the Pitié Salpêtrière Hospital (Paris). After years of collaboration, this consortium has received a 9 million euro subsidy from the Alzheimer Plan (Plan Alzheimer) to create a platform devoted to supporting multicentric neuroimaging studies. This platform draws on all the expertise available around France, concerning image analysis or acquisition optimization. It collaborates with the French radiology, neuroradiology and nuclear medicine societies. The essence of CATI lies in the networking of about fifty MRI and PET imagers spread over French territory. This network should be considered as a large instrument dedicated to the imaging of cohorts. This network of imagers reflects the French network of Memory Resource- and Research Centres (centres mémoires de ressources et de recherche) specializing in research into dementia. It will be extended in France and throughout Europe according to the needs of the users. The services proposed by CATI cover the entire multicentric neuroimaging chain. They more particularly concern the standardization of MRI, PET and SPECT (Single Photon Emission Computed Tomography) acquisition, secure data transfer to a centralized database, data quality control, data analysis *via* a portfolio of tools covering all imaging methods and data mining in order to identify biomarkers. The initial target for this platform was dementia, but it has since then been extended to research projects or therapeutic trials covering the entire spectrum of cerebral pathologies. It is today in charge of about fifteen French multicentric studies and an international therapeutic trial. CATI manages the imaging side for the Memento cohort of the Alzheimer Plan, the aim of which is to monitor more than 2,300 subjects recruited in the Memory Resource- and Research Centres.



G. Operto, C. Fischer, M. Perrot, D. Rivière and J.-F. Mangin

The human **cortex** folds extensively during the last three months of pregnancy, so as to increase its surface area while retaining our skulls within reasonable proportions. The patterns of these folds vary considerably from one individual to another. It is probable that abnormal developmental events leave a trace in these patterns, which should be possibly detected.

mining the large masses of data mentioned above. Thanks to these tools, the content of the images can be indexed, detecting the various structures of the brain. Nonetheless, traditional relational databases do not offer a simple means of managing the diversity of viewpoints among the neuroscience researchers, nor their constantly changing nature. New representation models are thus being developed. The computing power necessary for the complex algorithm tools used to analyse these masses of data implies the use of parallel computing, through dedicated computer clusters(2) and probably grid(3) or cloud(4) type architectures, something that the NeuroSpin teams are currently evaluating with the **NeugridForYou European Project**.

Constantly changing data

Although less breakneck than with the Internet, the pace of change in neuroimaging data nonetheless remains extremely rapid, because the longitudinal monitoring of patients is often far more sensitive in terms of diagnostics. Databases are no longer fixed, but evolve permanently. This is the case with the North-American project called Alzheimer's Disease Neuroimaging Initiative (ADNI), financed both by the NIHs and by the pharmaceutical industry and involving a thousand subjects suffering from early or late Alzheimer's disease. This database, which evolves permanently as data are acquired, is being used by hundreds of research groups around the world.

Discovering new biomarkers

In a world where diseases of the brain, whether neurodegenerative or psychiatric, take a heavy toll on our societies, big data offers new opportunities for discovering new **biomarkers** for including and monitoring patients in therapeutic trials. In recent years, considerable research work has shown that computer-based learning techniques(5) enable previously hidden patterns to emerge from data, patterns that had been invisible to the human mind and which represent true pathology signatures. These patterns could make early diagnosis possible, before the appearance of clinical signs, thus increasing the chances of success for the therapies. This is the role of imaging in the field of Alzheimer's disease, in which, after repeated failures of therapeutic trials on patients suffering from an advanced stage of the disease, the next attempts will target subjects who do not yet present precise symptoms, but for whom the brain images are indicative of Alzheimer's.

(2) Clusters: parallel computers.

(3) Grid: array of parallel computers originally designed for analysis of the data generated by the Cern.

(4) Cloud: generic computer power proposed by providers such as Amazon.

(5) Computer-based learning techniques: statistical methods enabling a computer to learn or discover patterns in a set of data.

> **Jean-François Mangin**
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Information Analysis and Processing Unit
Life Sciences Division
CEA Saclay Centre

Imaging, a link between **genetics** and **phenotype**

The severity of the handicaps inflicted by neurodegenerative and psychiatric diseases and their social burden have justified the launch of vast scientific research programs around the world. These programs aim to understand the biological mechanisms involved in these diseases and identify the biomarkers enabling them to be diagnosed and treated early on in the process. They are based on molecular biology and neuroimaging techniques.

Well before the appearance of molecular biology and neuroimaging techniques, linkage studies on the families of sick persons demonstrated the key role of **genetics** in the origin of neurodegenerative diseases – such as Parkinson's, early Alzheimer's – or psychiatric syndromes – such as schizophrenia, autism, major depression. In these studies, the patient or control status constitutes the sick **phenotype** and the baseline phenotype respectively.

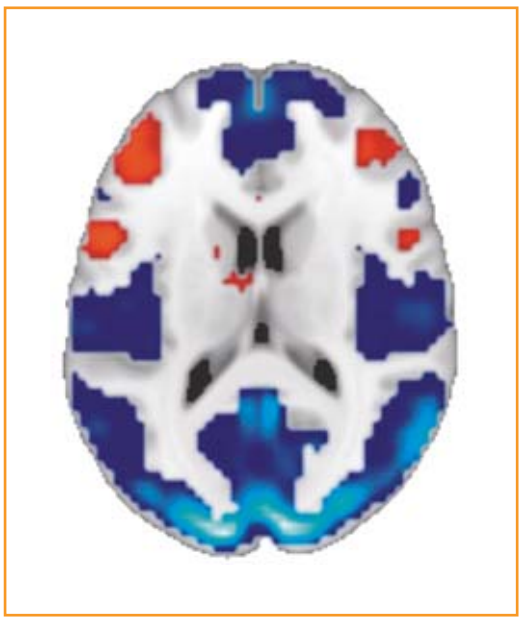
These diseases represent public health issues which justified the launch of extensive scientific work. Cellular biology experiments on animal models, consisting in deactivating the **genes** independently of each other, shed light on some of the biological mechanisms involved. They thus demonstrated that the risk of developing these diseases is the result of multiple and complex interactions between genes and environmental factors. However, these experiments only consider one gene at a time and their results are hard to transpose to humans.

Two major investigative tools

Two global investigative tools have recently opened up new prospects for research into neurodegenerative and psychiatric diseases: neuroimaging and molecular biology techniques.

Neuroimaging techniques – MRI for example – make it possible to conduct entire *in vivo* studies of the **central nervous system** of the patients, both anatomically and functionally. By comparing images obtained from patients with those obtained from the controls, regions showing anatomical or functional particularities were identified in the patients. Work in progress is thus helping to identify a number of candidate **biomarkers** for the diseases, the specificity of which has yet to be fine-tuned.

High-Throughput Sequencing technology (HTS), which has also become widely used in molecular biology, provides information supplementing that from neuroimaging. Based on the knowledge of the human **genome sequenced** in the late 90s, it uses **DNA chips** and high-speed sequencers. One of the possibilities offered by this tool is to reveal how the genome of one individual differs from that of another in identified, fixed regions called "genetic variants". These regions, which represent less than 1% of the whole genome, vary between individuals: this is known as the **genotype**. By comparing the genotypes of groups of patients with those of control groups, potential variants or sets of variants are identified *via* their particular configurations in the sick patients. Genes entailing a susceptibility to certain diseases have thus been identified through "candidate gene" studies, either by analysing a single variant at a time, or by a "genome-wide association" approach in which the state of all known variants of the genome is screened.



Ph. Pinel (Inserm – CEA U992), Y. Schwartz (Inria – CEA/NeuroSpin – Parietal) and V. Frouin (CEA/NeuroSpin)

The regions activated or deactivated by a calculation task performed by a subject are superimposed in false colours on the anatomy. These activated regions – acquired by functional MRI – can be considered to be phenotypes for genetic association studies.

A multidisciplinary approach

Conventional association studies, simply comprising the final phenotype and the genotype, require **cohorts** of patients and controls that are large enough and homogeneous enough for the phenotype to be correctly represented in them. In practice, the constitution of such cohorts is problematical. Firstly, when dealing with behavioural disorders, it is often hard to accurately identify the syndrome – and thus the phenotype – even if the batteries of behavioural tests are becoming more precise. In addition, there are

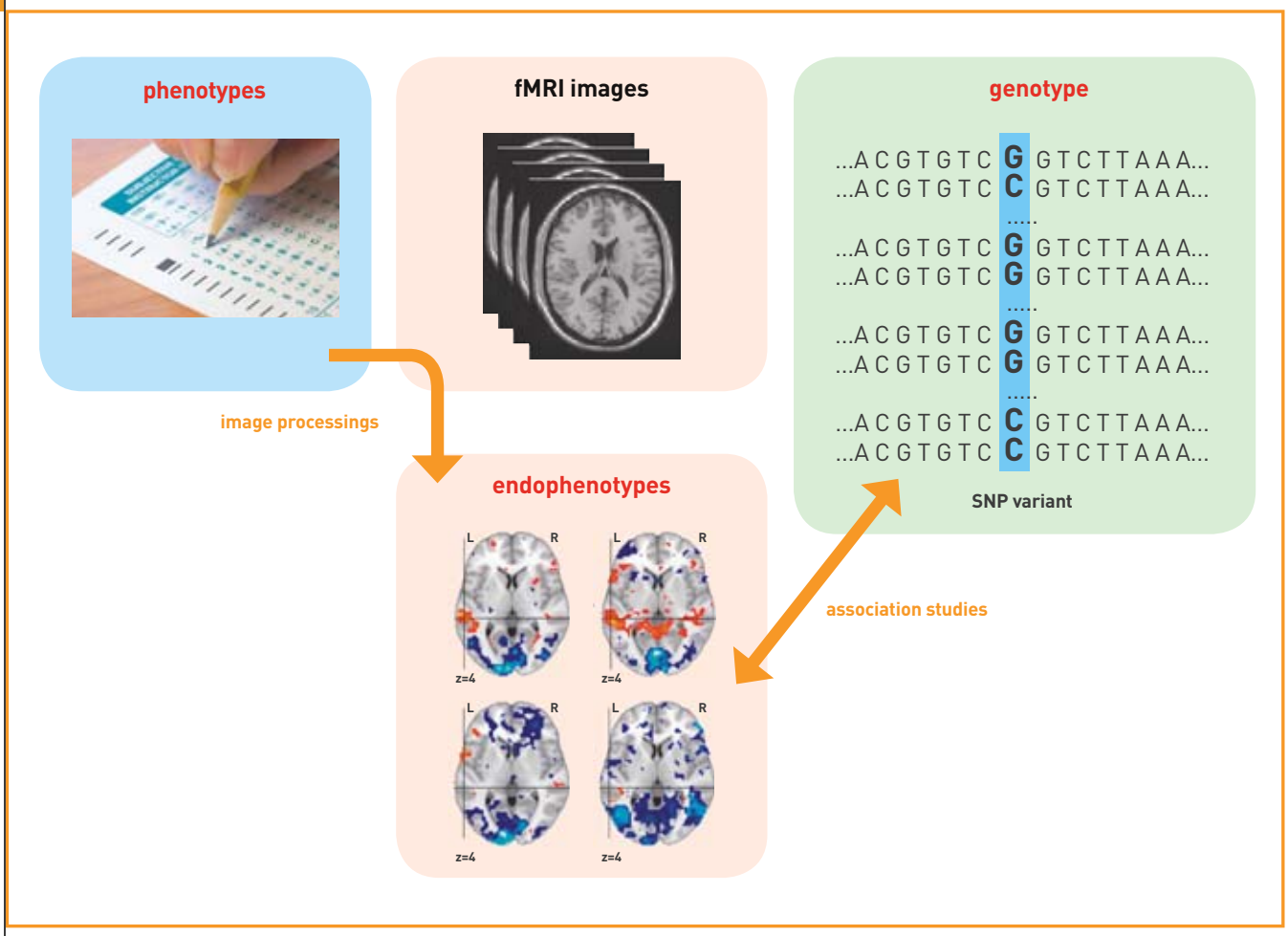


Figure 1.
Data acquired with imaging-genetics on patients and controls. In this example, the endophenotypes used are maps of regions activated for a cognitive task, obtained by functional MRI (fMRI). The genetic variants are of the Single-Nucleotide Polymorphism (SNP) type.

obviously no genes directly coding for the illness or the behaviour. The genes in question are mainly involved in the underlying biological mechanisms.

The idea at the heart of the "imaging-genetics" approaches is precisely, in addition to the overall phenotype, to study an *in vivo* phenotype closer to the actual biology, using the content of the images themselves. The biologists then talk of endophenotype. In practice, an imaging-genetics study involves three stages and is based on three types of measurements: the imaging data or endophenotype, the genotype data and finally the phenotype data taken from the clinical file or behavioural tests (figure 1).

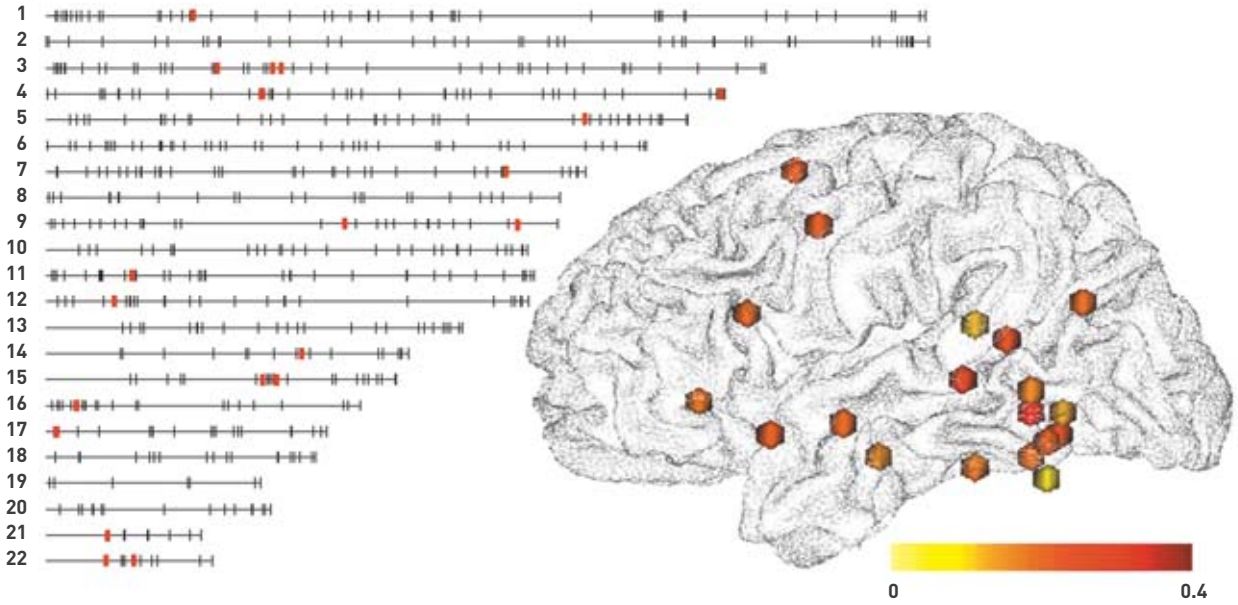
The first step involves the endophenotype: the images acquired in the various individuals are processed by image analysis in order to identify reliable individual characteristics, such as the shape and size of the sub-cortical structures, or grey matter density maps. Secondly, only those characteristics which distinguish the individuals according to their phenotype are selected. Finally, the last step consists in studying any associations between these characteristics of interest and the genetic variants.

The promise of imaging-genetics

The phenotype-endophenotype-genotype relays offered by imaging-genetics highlight the intrinsic potential of neuroimaging and genomic techniques. MRI provides endophenotypes of growing interest, such as the shape of the regions of the cortex or the basal ganglia or even how these areas are functionally involved in the performance of motor or cognitive tasks. For a given individual, MRI also makes it possible to construct the interaction networks between the various regions, at the anatomical (diffusion imaging) or functional (fMRI imaging at rest) levels.

As these endophenotypes evolve in the neurodegenerative diseases, imaging-genetics is an ideal tool for learning more by finding genetic associations. Neuroimaging, used in neuroscience, also allows the study of non-pathological phenotypes, such as reading learning scores (figure 2); see box on *Reading and genetic variants*. With regard to genomics, the new HTS techniques make it possible to study rare variants (complete sequencing) in an individual, or even the particular conformation (methylation) of certain DNA areas of his genome, determining the gene expression profile.

> **Vincent Frouin**
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Information Analysis and Processing Unit
Life Sciences Division
CEA Saclay Centre



Ph. Pinel (Inserm – CEA U992), E. Le Floch, V. Frouin and E. Duchesnay (CEA/NeuroSpin)

Figure 2.
Exploratory genomic study of associations between Single-Nucleotide Polymorphism (SNP) genetic variants and 19 regions differentially activated by a reading task. This imaging-genetics study concerns 100 control subjects. Activation is measured by functional MRI and the genotype is obtained by DNA chips. The method developed by the UNATI (Information Analysis and Processing Unit)/BrainOmics team allows isolation of a small number of SNPs (in red) which are associated with the activation of the areas involved in reading. Based on these results, it is possible to study the genes which contain these SNPs.

Reading and genetic variants

The importance of **genetics** in reading was first of all suggested by the recurring observation, within the same families, of several cases of patients suffering from dyslexia (troubles with learning to read). The comparison of reading score similarities between identical and fraternal twins then led to estimate that the share of genetic factors in the variability between individuals was approximately 50%. Finally, the genetic study of members of dyslexic families led to the isolation of a few **chromosome regions**, of varying sizes, in which the polymorphism was linked to the pathology, and to the identification of candidate **genes**.

Thanks to the use of **DNA chips**, it has become possible to fine-tune the location of these genetic variations. Studies conducted on several independent **cohorts** have led to the association of some **nucleotides** of chromosome 6, already known to modulate the expression of a gene involved in **neuronal** migration during development of the embryo (gene *KIAA0319*), at the risk of creating dyslexia. Even more interesting, their polymorphism also affects healthy subjects (good readers), both in terms of their behavioural scores and the degree of **lateralization** of their temporal lobe during reading tasks. The effects of other variants are beginning to be identified: for example, in the cell adhesion gene *CNTNAP2*, affecting the lateralization and the connectivity of areas of the brain that are important for reading (frontal and temporal regions), in its *FOXP2* regulation factor, with a more localized effect on the inferior frontal regions and the syntactic performance of baseline subjects, or in gene *ROBO1*, involved in establishing connections between the hemispheres, where the polymorphism apparently modulates the **phonological** memory. Even though learning to read involves educational and cultural factors, it is becoming increasingly clear that it is also influenced by genetic factors, which act at precise points of the biological support.

> **Philippe Pinel**
Institute of Biomedical Imaging (I2BM) – NeuroSpin
Inserm U992 – Cognitive Neuroimaging Unit
Life Sciences Division
CEA Saclay Centre

Gene variants and **structural ageing** of the brain

The incidence and prevalence of age-related diseases are constantly rising in our societies and it is therefore urgent to identify biomarkers able to predict their onset. Modifications in the volume and the morphology of certain brain structures have thus been correlated with the appearance of dementia in elderly subjects. The studies carried out on large cohorts of elderly patients not suffering from dementia are increasing what we know and enabling us to identify the genes causing these modifications.

PHOVOIR

In a context of increasing lifespan, the incidence of abnormal ageing brain diseases is rising. The pathological process however sometimes starts many years before the appearance of the first signs of the disease.

Current data are coming together to suggest that the pathological processes underlying various forms of dementia begin several years before the pathology is diagnosed. In healthy elderly subjects presenting no symptoms, it is possible to extract several **phenotypes** from the brain's structure, partly revealing these first infra-clinical changes. In this way, **biomarkers** of structural cerebral ageing such as the reduction in the total volume of the brain and the volume of the hippocampus, plus **white matter** hyperintensities have shown themselves to be powerful factors in predicting dementia and Alzheimer's disease.

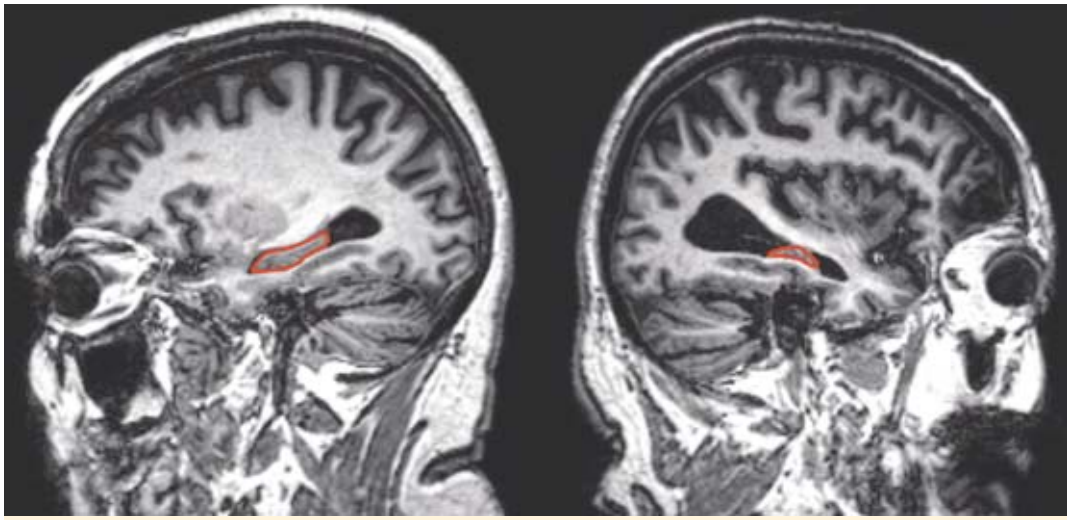
In a context of increased lifespan and the rising incidence and prevalence of age-related diseases, identifying risk factors has become crucial. This is in particular the case for the identification among the general population of **gene** variants associated both with structural cerebral ageing and an enhanced risk of dementia in elderly subjects not suffering from it. The discovery of these variants enables us to make progress in understanding the molecular mechanisms of neuroanatomical ageing, but also to make an early identification – based on their **genotype** – of certain individuals at risk of pathological ageing.

Atrophy of the hippocampus and the *ApoE* gene

Reductions in the volume of the brain and of the hippocampus appear with age under the cumulative effect of various factors. Atrophy of the hippocampus was a recognized biological marker for Alzheimer's disease and it is essential to determine the origin and more particularly the gene variants in this process, in order to more accurately decipher the mechanisms involved. Among these variants, the *epsilon-4* **allele** of the Apolipoprotein E ($ApoE^{epsilon4}$) **gene** has been the subject of extensive study for several years, because it is involved in the physiopathological processes of the brain. In particular, a correlation has been established between the presence of its *epsilon-4* isoform(1) and an increased risk of late-onset Alzheimer's disease, in parallel with discovery of a deleterious effect of $ApoE^{epsilon4}$ on the atrophy of the hippocampus.

Using **longitudinal relaxation time (T1) weighted** MRIs with millimetre **resolution**, and thanks to the use of automatic procedures (**Voxel-Based Morphometry**, VBM) specifically tailored to the study of structural ageing of the brain in elderly subjects, the researchers of the Neurofunctional Imaging Group [**GIN (Groupe d'imagerie neurofonctionnelle)** – UMR5296 CEA – **CNRS** and University of Bordeaux] measured the volume of

(1) ApoE, a lipid transport **protein**, exists in three major isoforms **coded** by three different alleles (3, 4 and 2).



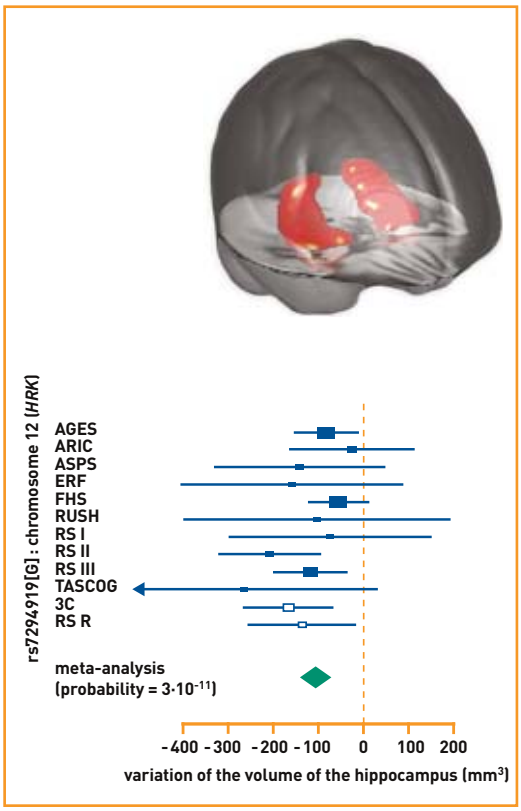
Inserm U1077-Caen/Francis Eustache

healthy subject

patient suffering from Alzheimer's disease

Magnetic Resonance Images (MRIs) of the brain of a healthy 80-year-old subject (left) and the brain of an 81-year-old patient suffering from Alzheimer's disease (right). The hippocampus appears in red. It is clear that the sick brain is atrophied. A detailed understanding of the mechanisms involved in this process, and in particular in the reduction of the volume of the hippocampus, which is a biological marker for this pathology, is therefore essential.

the brain and of the hippocampus non-invasively, quantitatively and reproducibly on very large population samples of elderly subjects, both transversely and longitudinally, in order to study the impact of this variant on these phenotypes.
First of all, on a **cohort** of 750 baseline subjects from 63 to 75 years old, from the EVA[2] survey, it was shown that subjects **homozygotic** for *ApoE*^{epsilon4} presented early atrophy of the hippocampus, predicting severe **cognitive** decline, measured seven years later. Conversely, no significant difference was observed between **heterozygotic** and non-carrier subjects for this allele. This work was reproduced and extended on the cohort of survey 3C[2], which includes a sub-sample of subjects homozygotic for *ApoE*^{epsilon4} and totally independent of the EVA base. By means of longitudinal monitoring of these subjects, it was possible to measure the change in the volume of the hippocampus for each of them. For a sample of 1,186 baseline subjects from 65 to 89 years old, who underwent two MRIs 3.6 years apart, a significantly higher ratio of atrophy in the volume of the hippocampus was revealed in the *ApoE*^{epsilon4} homozygotic subjects by comparison with the heterozygotic or non-carrier subjects. These results show that there is no **dose effect** for the *ApoE*^{epsilon4} allele and suggest that the impact of a single *epsilon-4* allele on the structures of the brain is considerably delayed (figure 1).



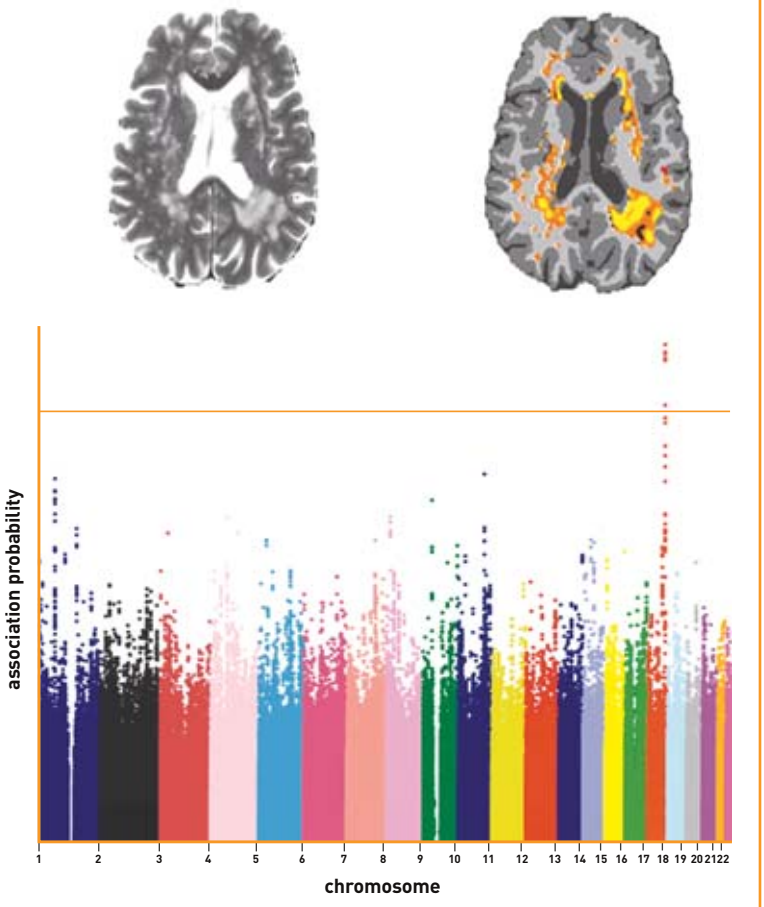
GIN

Figure 1.
The hippocampus (in red) is a structure buried deep within the brain. The GIN developed a method for analysing MRI images which, for each subject, allowed an automatic estimation of its volume. The diagram illustrates the 95% confidence intervals of associations between the volume of the hippocampus and a gene variant situated on chromosome 12 (*HRK* gene) for each discovery (blue rectangles) and replication (white rectangles) study by the international CHARGE[3] consortium. The association estimation by meta-analysis is here represented by a green diamond.

New gene variants

More recently, **genome-wide association studies**, examining not a disease but a target region of the brain, have identified several gene variants associated with a reduction in the volume of the hippocampus and/or the volume of the brain. These studies have been made possible thanks to the appearance of high-throughput **sequencing** and **genotyping** methods and the creation of international epidemiological study consortia in which the **genomes** and cerebral MRIs of thousands of subjects are pooled. In a study

(2) As of 1996, the GIN and U360 (A. Alpérovitch) then U708 (C. Tzourio) at **Inserm** contributed to the development of epidemiological neuroimaging research, with EVA and 3 Cities (3C) general cohorts. EVA is a transverse **monocentric study** of 844 subjects aged over 60, recruited from the general population of Nantes, in whom the risk factors for arterial and neuroanatomical ageing were studied. 3C is a longitudinal **multicentric study** (Dijon-Bordeaux-Montpellier) of a cohort of more than 2,500 subjects aged 65 and over.



CEA – CNRS – University Bordeaux Segalen/GIN

Figure 2.
At top left, volume cross-section of a transverse relaxation time (T2) weighted MRI in an elderly subject exhibiting a large white matter hyperintensity burden. At top right, result of individual and automatic detection of white matter hyperintensities. At bottom, the Manhattan plot illustrating the genome-wide association of the white matter hyperintensity burden. The probability of association is given according to the positions of all the *loci* tested on the whole genome. The horizontal line indicates the significant threshold for the whole genome. Only one *locus* on chromosome 17 presents a significant association with the white matter hyperintensity burden.

White matter hyperintensities

White matter hyperintensities are bright signals on **transverse relaxation time (T2) weighted** MRIs. Their morphology and their location vary. They appear in the form of spots or stripes of varying extents, or even converging clusters (figure 2). They are usually found close to the cerebral ventricles, but also deep in the white matter, or close to the **grey matter**. Depending on the clinical context, the white matter hyperintensities (bright signals) correspond either to the local destruction of **myelin**, or to a widening of the perivascular spaces. They are very frequent in elderly adults, where they are not necessarily the sign of a neuropathological process, but can however be the signature of an inflammatory disease, such as multiple sclerosis, or a vascular disease (small vessel disease in elderly subjects).

In this latter case, white matter hyperintensities could constitute a biomarker for cognitive decline associated with the vascular disease, predicting the onset of other ageing-related pathologies, or could be used to evaluate the efficacy of a therapeutic intervention. Studies of large cohorts, in particular the 3C survey, have been launched with the aim of studying the natural history of white matter hyperintensities and the factors associated with their occurrence and their development. In addition to the MRIs performed on several thousand subjects, this study also collected bioclinical, psychometric and genetic data.

One of the key objectives of this study was to see whether or not the presence of a high white matter hyperintensity burden was associated with the presence of certain gene variants. Thanks to a software developed by the GIN, the white matter hyperintensity burden of each of the subjects in the 3C survey was automatically calculated on each MRI and the data obtained were integrated with those acquired by the other members of the international CHARGE[3] consortium. It was thus possible to discover a new ***locus*** on **chromosome** 17 within which 6 gene variants were associated with a higher white matter hyperintensity burden. The discovery of these new variants, added to variant $ApoE^{epsilon4}$, is essential, as it will allow to obtain information on the molecular bases of an excessive burden of white matter hyperintensities, liable to lead to the development of therapies and better identification of the subjects at risk.

of 9,232 participants aged from 56 to 84, about 2,000 of whom belonged to the 3C survey, the variants associated with the reduction in the volume of the hippocampus concerned genes involved in numerous processes, including **apoptosis** (*HRK*), embryo development (*WIF1*), **oxidative stress** (*MSR3B*), **ubiquitination** (*FBXW8*), glucose tolerance (*DPP4*) and even **neuronal** migration (*ASTN2*). Even if we do not yet know whether the ***loci*** detected also influence cognitive decline and the risk of dementia, this study confirms that genetic factors are associated with a cerebral structure, the hippocampus, involved in dementia and more generally in the ageing of the brain.

(3) The international CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium) consortium was created to facilitate the study of genome-wide associations and the possibilities for replication between several large, correctly genotyped cohorts.

> **Fabrice Crivello** and **Bernard Mazoyer**
Institute of Biomedical Imaging (I2BM) – Neurofunctional Imaging Group (GIN)
Life Sciences Division

UMR5296 CEA – CNRS – University Bordeaux Segalen
Bordeaux

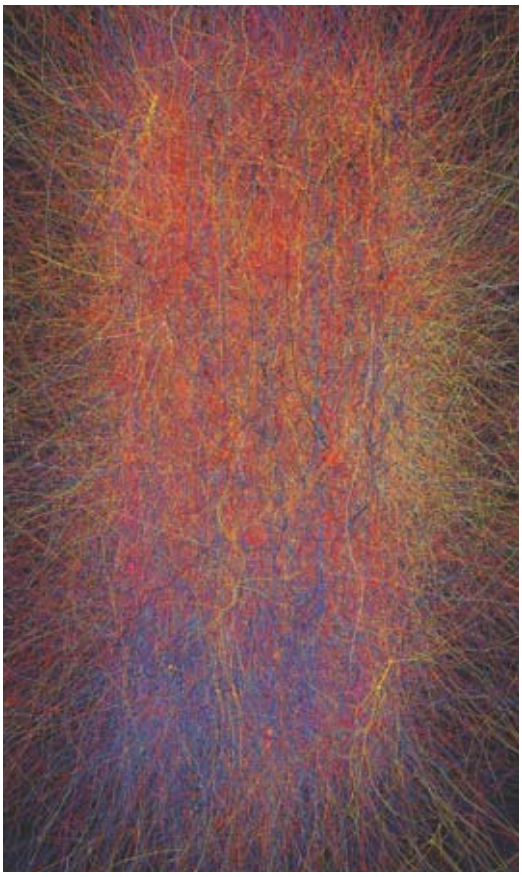
A **change in scale** for brain exploration

The scientific exploration of the brain is now entering a new era. A number of large-scale international projects are aiming to take advantage of the progress made in imaging and the information technologies in order to understand, model and simulate the working of the human brain, on all scales. Various projects have been launched almost simultaneously: the Human Brain Project in Europe, the BRAIN Initiative in the United States, the Brainetome in China. In addition to representing important advances in our theoretical knowledge, these major initiatives should lead to crucial applications in medicine, computing and robotics.

In January 2013, the European Commission officially designated the Human Brain Project (HBP) as one of the two winners of the European FET[(1)] Flagship program. The amount of the funding – 1.19 billion euros over ten years (2013-2023) – reflects the ambitions of the project: quite simply to model the working of the human brain... and create the technical means of achieving this. In the same year, the Obama administration launched the BRAIN (Brain Research through Advancing Innovative Neurotechnologies) initiative, aiming to map the activity of each **neuron** in the human brain – although starting with the simpler brains of certain animal models. China is also devoting considerable resources to its Brainetome project to explore and model the brain. The simultaneous emergence of these international initiatives represents a change in scale in the exploration of our "thinking machine". This should result in a giant leap forward for understanding the working of the brain, developing new diagnostics and therapies for brain diseases, but also information processing.

Ambitious projects

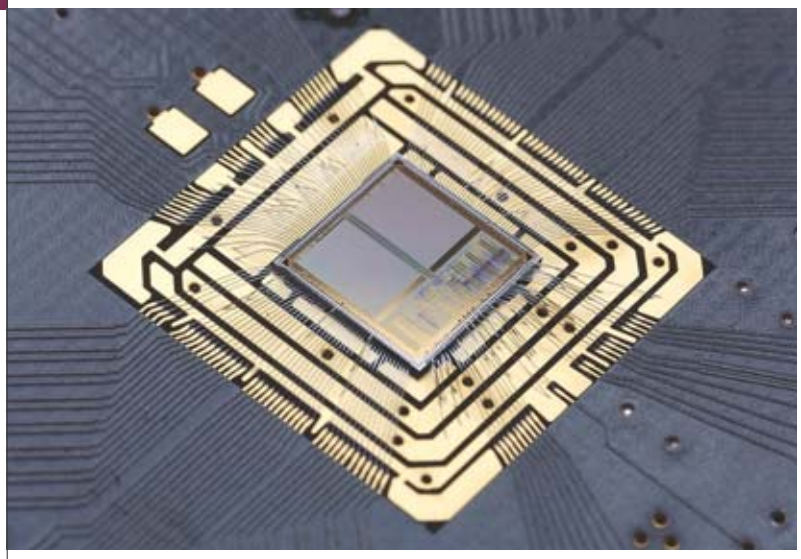
One common feature of these projects is that they involve the best specialists in the **cognitive** sciences, neuroscience, molecular biology, medicine, physics, mathematics, informatics and ethics. This is an essential interdisciplinary approach because the brain is today being described at different levels – **genetic** (more than a third of our **genes** are expressed in it), **synaptic** (role of the **neurotransmitters**), cellular (in particular with the organization of the neuron, one of the most complex human cells) – and at different anatomical scales (microcircuits consisting of networks of neurons, macrocircuits corresponding to the regions and areas of the brain)… Each of these aspects, studied by a specific scientific community, generates a phenomenal quantity of data, theories and algorithms. Hence the fundamental objective of these projects grouping together tens if not hundreds of laboratories, which is to collate these data of various types and integrate them into databases and functional models. With their help, the researchers will simulate in the slightest biological detail the processes employed by the human brain to process information, learn, feel, repair cell damage and so on. The degree of complexity of such modelling implies the design of specific software and the use of supercomputers, comprising an important mathematical and information processing aspect. Moreover, given the problems tackled here, ethical considerations are an integral part of the projects.



EPFL/Blue Brain Project

Modelling of a cortical column showing the complexity of the neuronal network. Apart from its layout in horizontal layers, the human cortex is organized in vertical functional units called cortical columns. The neurons on a column respond to the same type of stimulation.

(1) Future and Emerging Technologies. The other winner is the Graphene program.



Heidelberg University, Germany

Neuromorphic chip developed at Heidelberg University in Germany by the team of Karlheinz Meier. It simulates 384 neurons, 100,000 synapses and processes information in real time 100,000 times faster than the human brain.

The "google-map" of the brain

If we take the example of the HBP, the project will call on equipment distributed over six platforms. The *Brain Simulation Platform* will run various brain functioning models at high speeds. The goal of the *Neuromorphic Computing Platform* is to "translate" these models into ultra-fast electronic chips, called neuromorphic chips, which simulate the neurons and their connections. They will be usable to create realistic models at different scales. For example, a laboratory wishing to test a new drug will opt for simulation at the neurotransmitter scale. The *Neuroinformatics Platform* will be open to all countries, including those which are not members of the HBP, and will create databases containing all available knowledge about the brain. The challenge is to understand the **cortical** maps and the **neural code** associated with each of them. This platform could become a sort of "google-map" of the brain, making it possible to zoom in and collect quantitative data at different scales.

High performance and low consumption

The *High Performance Computing Platform* will propose large computers for cerebral simulation. Within the next ten years there are plans to develop a supercomputer capable of achieving the "exascale"(2) speeds required for certain simulations. As its name suggests, the *Medical Informatics Platform* will collate clinical data on the human brain and apply "Knowledge Discovery in Databases" (KDD) algorithms to them, in order to redraw the landscape of cerebral pathologies. The hoped for aim is to identify homogeneous patient groups corresponding to a new definition of the diseases, relying on sets of **biological markers**. This part of the project will to a large extent be built around cerebral imaging. In order to utilize the largest possible databases, hospitals will be encouraged to share theirs in complete anonymity. Finally, the purpose of the *Neurorobotics Platform* is to implement in robots the knowledge obtained in the human brain. One key objective for neuromorphic chips in particular is to perform calculations using very little energy, in the same way as the brain. This latter only consumes 20 watts to perform far more operations than a supercomputer draining 20 megawatts!

(2) Exa is the prefix of the international system of units representing 10^{18}. The computing speed will be about one billion billion operations per second.

French participation

CEA, **Inserm**, **CNRS**, **Inria**, the Pasteur Institute (**Institut Pasteur**), the **Collège de France** and the Victor Segalen Bordeaux II University are all participants in the HBP, in particular coordinating three areas of the first phase of the project: the theory of neuronal networks (Alain Destexhe, CNRS), ethical aspects (Jean-Pierre Changeux, *Collège de France*, Pasteur Institute) and cognitive neuroscience (Stanislas Dehaene, *Collège de France*, Inserm, CEA). A second step of the project, already under preparation, will be starting in 2017.

A new Institute

The recent creation of the European Institute for Theoretical Neuroscience (EITN), installed in the Paris area owing to the extensive local theoretical and mathematical community, is part of the HBP. This institute is destined to become a crossroads for the various theoretical channels concerning brain dynamics, the emergence of consciousness and cognitive processes. It will play an essential role in the search for neuronal coding mechanisms, based both on experimental data and numerical simulations and on the deployment of these mechanisms in neuromorphic circuits. It is currently being housed by the Paris Vision Institute (*Institut de la vision*), but will eventually move into the building of the Neuroscience Paris-Saclay Institute (*Institut des neurosciences*), on the CEA site.

By encouraging a continuous process of interdisciplinary integration and iteration, the HBP and the other major international initiatives should open the door to a unified understanding of the mechanisms and principles underpinning the working of the brain. Their modelling will no doubt lead to new tools for the diagnosis and treatment, in particular pharmacological, of certain neurological or psychiatric diseases, as well as innovative prosthetic technologies to help the disabled. In a completely different field, improved understanding of how the brain works should have spin-offs for informatics and robotics, in particular inspiring the design of future computers.

> **Anne Flüry-Hérard**[1]
and **Jean-François Mangin**[2]
[1]Institute of Biomedical Imaging (I2BM) – Dir
CEA-Frédéric Joliot Hospital Service
Life Sciences Division
Orsay

[2]Institute of Biomedical Imaging (I2BM) – NeuroSpin
Information Analysis and Processing Unit
Life Sciences Division
CEA Saclay Centre

Institutions and organizations: who does what?

(this list only presents the institutions, organizations and projects mentioned in the articles)

Aalto University: University of Helsinki (Finland) proposing studies in engineering technology and science, business and economics, art, architecture and design. It was created in 2010 and comprises six higher education establishments and a number of units and institutes. It in particular focuses on biomedical engineering and computational science.

AFM-Téléthon (Association française contre les myopathies): the French Muscular Dystrophy Association is an association whose main role is to provide funding, obtained from donations made during a "TV marathon", for research projects into rare genetic diseases, including neurodegenerative genetic diseases.

AIM (Association Institut de myologie): the purpose of the French Institute of Myology, created in 1996 by AFM-Téléthon, is to promote and raise the profile of myology. It is a reference centre for the diagnosis, treatment and monitoring of neuromuscular diseases. It is also an applied and fundamental clinical research infrastructure and a centre for training and for the spreading of myology.

AP-HP (Assistance publique-Hôpitaux de Paris): this University Hospital Centre for the Île-de-France region is the leading CHU (*Centre hospitalo-universitaire*) in Europe. With 37 hospitals gathered in 12 hospital groups and a multi-disability federation, the AP-HP proposes care and treatment modes tailored to local health needs. It also devotes its full expertise and its innovative capabilities to patients affected by complex pathologies.

ATGC: a bioinformatics platform centred in Montpellier (Hérault *département*), belonging to the French Institute of Bioinformatics (IFB: *Institut français de bioinformatique*), which replaced in December 2014 the ReNaBi network. It comprises six laboratories located in South of France and working on phylogenetics, population genetics, molecular evolution, genome dynamics, comparative and functional genomics, and transcriptome analysis.

Ceregene: a bio-engineering firm in San Diego (California, United States) specializing in the treatment of Parkinson's and Alzheimer's diseases (by simultaneously using gene therapy – AAV-2 viral vectors – and neurotrophic factors).

Clinatec: a multimodal translational technology research platform, comprising physicians, biologists and technologists, designed to validate innovative strategies derived from micro-nano-technologies and electronics. This biomedical research centre was developed by CEA/Leti in partnership with the Grenoble University Hospital (CHU), Joseph Fourier University and Inserm and meets major medical needs in the fields of cancer, neurodegenerative diseases and disability.

CNRS (Centre national de la recherche scientifique): the French National Centre for Scientific Research is a public-sector establishment of scientific and technological character, carrying out its activity in all fields of research. The CNRS devotes two interdisciplinary commissions to neuroscience: "Health and Society" and "Cognition, language, information processing, natural and artificial systems". Two fields, linked to the analysis of the nervous system complexity, benefit from this: neuroinformatics and modelling. Furthermore, in the field of physiopathology, the crossover between disciplines opens up therapeutic prospects in neuropsychiatry, through the definition of innovative strategies be they pharmacological or, further into the future, those relying on new assistance systems: robotics, nanotechnologies, microstimulation.

Collège de France: founded in 1530 by King Francis I, the Collège de France is a public higher education institution that is unique in France and has no equivalent abroad. It is a place where research is both carried out and taught. Today, 57 professors cover a vast range of disciplines from mathematics to the study of major civilizations, through physics, chemistry, biology, medicine... The recently-created Chair of Experimental Cognitive Psychology is occupied by Stanislas Dehaene (CEA/NeuroSpin). It is devoted to studying the impact on the brain of learning written symbols (numbers, words) and to studying consciousness.

Ernst Strüngmann Institute: an institute located in Frankfurt (Germany) and devoted to neuroscience in cooperation with the Max Planck Society. This private institute has set itself the goal of producing high-quality fundamental brain research. This search focuses on the contribution of the different parts of the brain to human behaviour.

Euro-BioImaging: a pan-European infrastructure which is among the ten biological and medical science projects included in the European Strategy Forum on Research Infrastructures (ESFRI) roadmap. It aims to bring together the main fields of biological and medical imaging. Euro-BioImaging gives access to training in imaging technologies and to sharing of best practices and of a biological image database. It is a tool that helps drive European innovation in biological and medical imaging.

Généthon: an organization created in 1990 by the French Muscular Dystrophy Association (AFM-Téléthon) and the French Human Polymorphism Study Centre (CEPH: *Centre d'étude du polymorphisme humain*). Although initially founded to develop the tools essential to the understanding of genetic diseases, Généthon today contributes to the development of gene therapies for rare diseases.

GIN (Groupe d'imagerie neurofonctionnelle): founded in 1989 by a group of researchers at CEA/SHFJ, the Neurofunctional Imaging Group has become a pioneer in France in the development of Positron Emission Tomography (PET) and its use for investigating the neural bases of cognition. Over time, it has acquired know-how in all the functional imaging techniques (PET, MRI, EEG, MEG). In 2000, it became a CEA – CNRS Joint Research Unit of the University of Caen (Calvados *département*) Basse-Normandie and in 2011, it joined the University of Bordeaux (Gironde *département*). The GIN in particular studies the key phenomena in the organization of the human brain, by relying on the building and/or analysis of large multimodal databases combining psychometric, neuroanatomical, neurofunctional and genetic measurements.

ICM (Institut du cerveau et de la moelle épinière): the French Brain and Spine Institute is a private foundation recognized as being in the public interest, located in the Pitié Salpêtrière Hospital and specializing in diseases of the nervous system (see Institut hospitalo-universitaire de neurosciences translationnelles de la Pitié Salpêtrière).

Imagen European Project: a research project set up by the European Commission in 2002 (6th Framework Program for Research and Technological Development), focusing on risk-taking behaviour in teenagers. It is coordinated by the Institute of Psychiatry King's College London (Great Britain). Seventeen organizations, including CEA and Inserm, from 5 countries, are participants. The work performed concerns behavioural studies, interviews, brain neuroimaging and genetic analyses.

INESC-MN (Instituto de Engenharia de Sistemas e Computadores-Microsistemas e Nanotecnologias – System and IT Engineering Institute-Microsystems and Nanotechnologies): a private, non-profit research and development institute situated in Lisbon (Portugal) and specializing in micro-nano-technologies and their applications in electronics and in medical and biomedical devices.

Inria (Institut de recherche en informatique et en automatique): the French National Institute for Research in Computer Science and Control is a public scientific and technological research institute founded in 1967. Inria comprises eight centres and its role is to produce world-class research in the IT and mathematical fields of digital science and to guarantee the impact of this research. The Inria Saclay Île-de-France centre directs its research in three directions: software security and reliability, high-performance computing, and modelling, simulation and optimization of dynamical complex systems. This latter topic has applications in the processing of medical images and in understanding the working of the brain.

Inserm (Institut national de la santé et de la recherche médicale): the French National Institute of Health and Medical Research is a public scientific and technological research institution placed under the dual supervision of the Ministry for Social Affairs, Health and Women's Rights, and the Ministry for National Education, Higher Education and Research. It is the only French public research organization entirely dedicated to human health. In 2008, Inserm was given responsibility for strategic and operational coordination of biomedical research. It performs translational research, from the laboratory to the patient's bedside. Expert assessments and scientific intelligence are also part of its official duties. Ten Multi-Organization Thematic Institutes (ITMO) placed under the authority of the French National Alliance for Life Sciences and Health (Aviesan) [created by Inserm, CNRS, CEA, Inra, Inria, IRD, the Conference of University Presidents and the Institut Pasteur] were thus set up with clearly defined objectives.

Institut hospitalo-universitaire de neurosciences translationnelles de la Pitié Salpêtrière: the Pitié Salpêtrière Research Institute for Translational Neuroscience (IHU-A-ICM) is a scientific cooperation foundation combining the scientific and medical expertise of four public (AP-HP, CNRS, Inserm, Pierre et Marie Curie University) and private partners. Its role is to develop new therapeutic strategies in order to improve treatment and to slow down or repair lesions caused by nervous system diseases. Its research activity is grouped at ICM which offers a unique environment combining technology platforms, an industrial workspace and a clinical investigation centre.

Institut Pasteur: a French private, non-profit foundation devoted to the study of biology, micro-organisms, diseases and vaccines. This organization was set up in 1887 by Louis Pasteur and was initially dedicated to treating rabies, conducting research into infectious diseases and teaching. The Institut Pasteur is currently an international reference in terms of infectious diseases and has broadened its scope of activity to include neuroscience, development biology, stem cells, genetics and genomics.

I-Stem (Institut des cellules souches pour le traitement et l'étude des maladies monogéniques): the Institute for Stem cell Therapy and Exploration of Monogenic diseases was created in 2005 under a collaboration between Inserm and AFM-Téléthon. It specializes in research and development on pluripotent human stem cells from embryos or obtained by genetic reprogramming. I-Stem is a member of the Biotherapies Institute for Rare Diseases, which currently comprises the four research and development centres directly financed by AFM-Téléthon.

MIRCen (Molecular Imaging Research Centre): a research infrastructure dedicated to the development of translational methods and assessment of the therapeutic efficacy of biotherapies, primarily in the field of the neurodegenerative diseases, but also infections and cardiac diseases. This infrastructure is established in CEA's Fontenay-aux-Roses centre (Hauts-de-Seine *département*), and is a joint CEA and Inserm research centre. CNRS also makes an essential contribution through a Joint Research Unit. MIRCen has enjoyed longstanding and fruitful collaboration with Henri Mondor (Créteil, Val-de-Marne *département*) and Pitié Salpêtrière (Paris) hospitals. This installation consists of a group of platforms devoted to developing animal models representative of human pathologies used to design innovative therapies. MIRCen is closely involved in the "NeurATRIS" and "France Life Imaging" national biology and health infrastructures which follow the line of the major European infrastructures.

NeugridForYou European Project: a project set up by the European Commission (7th Framework Program for Research and Technological Development) and financed by its Directorate-General, with the aim of extending and improving the use by researchers of the Neurogrid web portal. Neurogrid is dedicated to research into neurodegenerative diseases and gives researchers access to MRI imaging archives, analysis algorithms and statistical tools.

NeuroSpin: a centre for cerebral neuroimaging by intense field nuclear magnetic resonance, set up in 2007 in the CEA Saclay centre (Essonne *département*). NeuroSpin operates large MRI imaging instruments producing magnetic fields of 3 T, 7 T, 11.7 T (in 2015) and 17.2 T (studies on small animals) as well as an MEG device. On a single site, this research infrastructure accommodates imaging and signal processing methodologists and neurobiologists in order to improve our understanding of the working of the normal and pathological brain. NeuroSpin in particular takes part at the French level in the "Neuroscience and physics-medicine interface" project, within the framework of the Paris-Saclay University Campus, in the "France Life Imaging" and "NeurATRIS" biology and health infrastructures which follow the line of the major European infrastructures, and in the Human Brain Project, the aim of which is to model the human brain.

NeuroStemCell: this research consortium was set up by the European Commission (7th Framework Program for Research and Technological Development) and its objective is to develop therapies using stem cells, for Parkinson's and Huntington's diseases. It comprises partners, including CEA and Inserm, from 6 European countries and the United States.

Plan Alzheimer: the French Government's five-year Alzheimer Plan, set up in 2008, with the aim of organizing and coordinating the overall care picture for persons affected by Alzheimer's disease and their carers. Specific resources were implemented, based on 44 concrete measures in three areas: "Improving quality of life for patients and carers", "Knowledge for action" and "Mobilizing around a social issue". An evaluation report was published in June 2013 and the extension of the plan was announced in September 2013.

Programme d'investissements d'avenir (PIA): the Investing in the Future Program is financed by the major national loan launched on the financial markets in 2010 and is worth 35 billion Euros. It aims to modernize and boost the competitiveness of France, by promoting investment and innovation in five priority sectors: higher education and training, research, industrial sectors and SMEs, sustainable development and the digital sector.

Service Hospitalier Frédéric Joliot (SHFJ): created in 1958 by CEA in order to develop nuclear medicine, Frédéric Joliot Hospital Service is today a centre for molecular and functional non-invasive imaging, dedicated to pre-clinical and clinical research. It is located in Orsay (Essonne *département*), and houses joint CEA - CNRS and CEA - Inserm teams. With its teams specializing in methodology and processing of PET and MRI images, the SHFJ proposes a multimodal biomedical imaging approach in the fields of neurology, psychiatry and oncology. Particular focus is given to the development and validation of imaging biomarkers. The SHFJ also functions as a hospital, being the nuclear medicine unit of the Orsay hospital centre.

TRANSEURO: set up by the European Commission (7th Framework Program for Research and Technological Development), TRANSEURO is a research consortium whose main goal is to develop an effective and safe treatment methodology for Parkinson's disease using foetal cell-based treatments. It has gathered international experts including clinicians, scientists, industrial partners, ethicists and patients' representatives.

UNIC-CNRS (Unité de neurosciences, information et complexité): situated in Gif-sur-Yvette (Essonne *département*), the Unit of Neuroscience, Information and Complexity is one of the four units making up the Institute of Neurobiology Alfred Fessard (INAF). It adopts interdisciplinary approaches and one of its scientific aims is to characterize the complexity of biological systems. It in particular studies the complexity of the neocortex during sensorial perception throughout its development. The UNIC unit is expected to join the Neuroscience Paris-Saclay Institute.

Glossary

A

Adeno-Associated Virus (AAV): a small DNA virus that is generally not pathogenic. AAVs are often used in gene therapy as gene transfer vectors.

adenoviruses: a family of about a hundred viruses possessing linear double-stranded DNA. Of them, forty or so varieties can infect the human species and cause pathologies that are primarily respiratory, but also ocular and in certain cases digestive.

adrenaline: a neurotransmitter and hormone of the same family as noradrenaline and dopamine. It is secreted in the event of stress, accelerates cardiac activity and increases blood pressure. It is synthesized suddenly to help the organism deal with a threat.

adrenergic (neurons): neurons for which the neurotransmitter is adrenaline.

allele: one of the different versions of a given gene or a given *locus*. The alleles contribute to the genetic polymorphism.

amino acid: an organic molecule containing an amine group ($-NH_2$) and a carboxyl group (–COOH). Twenty different amino acids, the sequencing of which is coded by DNA, are the basic components of proteins.

amyloid plaque: an extracellular accumulation of beta-amyloid forming pathological aggregates. This is one of the two lesions characteristic of Alzheimer's disease.

aneurysm: a localized dilation of the wall of an artery, leading to the creation of a bulge or a significant increase in its diameter.

angioma: a malformation resulting from the dilation of blood or lymph vessels (vessels transporting the lymph, which consists of blood plasma and white blood cells).

anion: an atom or a group of atoms carrying one or more negative charges.

anisotropy: characteristic of a phenomenon or a system for which one property depends on its spatial orientation.

anisotropy (in diffusion MRI): diffusion MRI allows to measure the distribution of water molecule movements. In the brain, this distribution is anisotropic and can be used to identify the tracts of axons in the white matter. Modifications of this anisotropy are studied in certain illnesses such as schizophrenia.

apoptosis: a process of programmed cell death, necessary for the survival of multi-cell organisms, in which certain excess cells organize their own destruction in response to a given signal.

astrocytes: glial cells which play an active role in maintaining the blood-brain barrier, and in providing metabolic support and glucose nutrients to the neurons (*type-I astrocytes*). They are also involved in the propagation of the nerve signals by acting on the release and reuptake of the neurotransmitters (*type-II astrocytes*).

axon: fibrous extension of the neuron carrying the nerve impulse.

B

basal ganglia: part of the grey matter situated in the centre of the central nervous system and organized into neuronal groups (nuclei). They are directly linked to all the regions of the cortex – except for the primary sensorial areas (sight, touch, smell) – and take part in the motor, affective and cognitive functions. Their dysfunction is involved in various neurodegenerative diseases such as Parkinson's disease.

biomarker: the signature of a normal or pathological biological process one is seeking to reveal.

blood-brain barrier: the physiological cell barrier separating the Central Nervous System (CNS) from the blood. It protects it from the various agents or circulating cells present on the periphery, while being selectively permeable to the nutrients and oxygen the brain needs and to the CNS waste which it allows to be eliminated.

C

^{11}C-raclopride: raclopride is a compound of the benzamide class acting as a dopamine D_2-receptors antagonist, which exhibits antipsychotic properties. When labelled with carbon 11, it is used in Positron Emission Tomography (PET) to evaluate the quantity of dopamine bound to the D_2-receptors or the disappearance of the neurons carrying these receptors. It is also used to determine the efficacy and neurotoxicity of dopaminergic system drugs.

carbon nanotube: a crystalline structure consisting of one or more rolled up graphene sheet(s) (2D assembly of carbon atoms arranged in a hexagonal array; it is a conductor with unique electrical properties), with a nanometric diameter but with a length that can range from 100 nm to a few millimetres. Carbon nanotubes are excellent electrical and thermal conductors and also exhibit remarkable mechanical and optical properties.

catalysis: a process involving a substance (the catalyst) capable of accelerating a chemical reaction by modifying its mechanism. In principle, the catalyst is not consumed and is restored at the end of the reaction. In biology, most catalysts are enzymes (enzymatic catalysis).

catecholamine: a family of neurotransmitters whose molecule consists of a benzene ring carrying two hydroxyl groups (–OH) and an amine group ($-NR_1R_2$). Dopamine, adrenaline and noradrenaline are catecholamines.

cation: an atom or a group of atoms carrying one or more positive charges.

centres mémoires de ressources et de recherche: at the request of general practitioners or memory units, the Memory Resource- and Research Centres receive patients whose troubles require in-depth assessment. These centres animate the French regional network of memory units and conduct research work in this field.

cerebral connectomics: the set of connections established between the neurons of the brain.

cholinergic (neurons): neurons for which the neurotransmitter is acetylcholine. Acetylcholine is involved in the central nervous system where it in particular takes part in the learning and memorization processes. It also plays a role in the peripheral nervous system (muscle activity for example).

chromatin: the form of DNA inside the nucleus of a eukaryotic cell. It is condensed into chromosomes at the beginning of mitosis (one of the phases of cell division).

chromosome: the molecular medium carrying genetic information. It consists of DNA molecules and proteins. The chromosomes are to be found in the nucleus of eukaryotic cells and in the cytoplasm of prokaryotic cells (nucleoid). They are observable in the condensed form in the cells only for a short period of the cell cycle preceding their division. The genes cannot express at this moment. Between two divisions, the DNA is decondensed and forms the chromatin within which gene expression takes place.

chromosome (region): each chromosome is divided into arms (long or short) situated on either side of the centromere. These arms themselves comprise regions divided into bands and sub-bands. This enables each gene to be precisely located according to a code.

clinical (study or research): a study performed in human medical therapeutics to assess the efficacy of a diagnostic method or of a treatment on a panel of patients.

code, to: each gene which expresses originates the synthesis of a protein *via* an RNA. The gene (or allele) is said to code for this protein.

cognition: the set of mental processes relating to knowledge. These processes were originally split into various functions (perception, language, memory, reasoning, executive functions, emotions, etc.), but are closely interconnected. Its study is the subject of the cognitive sciences.

cohort: a group of individuals specifically chosen for a statistical study.

complexation (of DNA): the outer part of the DNA molecule consists of phosphate groups, each of which exhibiting a negative charge: DNA is a polyanionic molecule. It is thus liable to be surrounded by cations (such as certain lipids) to form a complex.

computational science: part of the sciences resorting to mathematical models and high performance computing. It is used to perform simulations and to study complex systems for which experimentation cannot be envisaged.

computations (neural): logic operations performed by the neurons and comparable to IT computations.

contrast agent: a compound which, when present in a structure, increases the contrast of a medical image, thus making it possible to visualize this anatomical (such as blood vessels) or pathological (for example a tumor) structure which naturally has little or no contrast and which would therefore be hard to differentiate from the surrounding tissues.

cortex: the outer layer of an organic tissue and more particularly of the brain. The cerebral cortex, consisting of a layer of grey matter situated on the surface of the hemispheres, contains the neuron cell bodies and is the site of the highest cognitive functions.

cytokine: a protein or glycoprotein used for communication between the cells of a living organism. The CNTF (Ciliary Neuro-Trophic Factor) cytokine, synthesized by the astrocytes and the Schwann cells, exhibits neuroprotective effects.

D

dendrites: branched extensions of the neurons contributing to their interconnection. Dendrites receive nerve impulse on specialized organelles called synapses and transmit them to the cell body.

differentiation (cellular): a process in the development of living organisms which triggers the transformation of non-specialized (pluripotent) stem cells into highly-specialized cells, the properties of which are defined by their environment and their location.

Diffuse Correlation Spectroscopy (DCS): a process used to measure blood flow by means of infrared light and the Doppler effect. This method is non-invasive and allows investigations to a depth of about one centimetre and a half.

diffusivity (in diffusion MRI): the property of a chemical species to propagate in a given direction. Diffusion MRI allows to measure the diffusivity of the water molecules which, for the neurons, is greater along the axons (axial diffusivity) than in the direction perpendicular to their membrane (radial diffusivity).

DNA: DeoxyriboNucleic Acid. Essential component of chromosomes, this molecule carries the genetic information within the living cell (genes are DNA segments). DNA is formed of a double chain of nucleotides the bases of which can be adenine (A), guanine (G), cytosine (C) or thymine (T). Each strand is connected to the other by hydrogen bonds, with A–T and C–G correspondence.

DNA chip: a device based on microelectronics techniques and used to detect the presence of a strand of DNA. This one matches with its complementary DNA strand fixed on the chip, on hybridization sites according to the DNA double helix principle. For example, this method makes it possible to analyse the global expression of the genes in a cell.

DNA sequencer: a device capable of performing automated DNA sequencing.

dopamine: a neurotransmitter and hormone of the same family as adrenaline and noradrenaline of which it is the precursor. It is produced mainly by the neurons of the substantia nigra (present in the basal ganglia) and the ventral tegmental area (belonging to the reward system) and plays an essential modulator role in motricity, attentional processes and addictive phenomena.

Doppler effect: the frequency of a wave differs at its emission and at its reception if a relative movement exists between the emitter and the receiver. The shift measured, which depends on the relative speed between emitter and receiver, is the Doppler effect. It is used in echography or in Diffuse Correlation Spectroscopy to measure blood flows.

dose effect (of an allele): an allele can be replicated several times in the genome of a living organism. Its expression is thus linked to the number of these replicas: this is the dose effect.

dystonia: motor disorder of neurological origin inducing involuntary, prolonged muscular contractions leading to abnormal postures or movements.

E

enzyme: a molecule able to reduce the activation energy of a reaction and to accelerate it up to several million times without modifying the resulting equilibrium. The chemical reactions of the metabolism taking place in the cellular or extracellular medium are thus facilitated and accelerated by these proteins, which are the catalysts of the living world.

etiology: the set of causes and factors characteristic of a given pathology.

eukaryotic: refers to single- or multi-cell living organisms which are characterized by the presence of a nucleus and mitochondria in their cells.

F

fovea: the central part of the macula which, on the retina, is situated in the visual axis of the eye. It consists solely of cones and provides excellent spatial resolution.

fragile X syndrome: a chromosome anomaly due to a genetic mutation situated on the *FMR1* gene of the X chromosome, and/or on either side of this gene. Several visible signs allow a probable diagnosis (elongated face, large or protruding ears, prominent forehead and jaw, numerous mental disabilities). There is no treatment other than adaptation to behavioural disorders.

free radicals: atoms or groups of atoms which can still establish one or more chemical bonds with other atoms. They are involved in chemical reaction mechanisms. In biology, they are powerful reagents which can disrupt the equilibrium of living cells (oxidative stress).

Friedreich's ataxia: a neurodegenerative disease characterized by balance and speech disorders. It is accompanied by cardiac troubles and sometimes diabetes. It is caused by the mutation of the *FRDA* gene on q13 *locus* of chromosome 9.

G

GABAergic (neurons): neurons for which the neurotransmitter is γ-aminobutyric acid (or GABA). This is an inhibitory neurotransmitter derived from glutamic acid.

gamma photon: a quantum associated with the electromagnetic field and whose wavelength is smaller than 0.1 nm (10^{-10} m). They are generally produced by nuclear processes at the heart of nuclei or by violent events in the Universe (gamma ray bursts). Their energy is more than a few keV and can exceed ten GeV. The 511-keV photons resulting from the annihilation of an electron-positron pair are also called gamma photons, and their detection is utilized in Positron Emission Tomography (PET).

gene: a hereditary information unit consisting of a string of nucleotides and written in the DNA or RNA. DNA gene expression requires the production of an RNA which ensures the synthesis of a protein. This one then takes part in the functioning and determination of the characteristics of the cell which contains it.

genetics: a part of the life sciences studying the transmission and expression of genes at the cellular level, but also at the level of the organism and species.

genome: the set of genes of a living organism.

genome-wide association study: a statistical study designed to discover the genes involved in a given phenotype. This study identifies which, among a large number of genes, are significantly present in or absent from a cohort of subjects who exhibit the studied phenotype by comparison with another cohort of subjects who do not.

genotype: information carried by the genome of a living organism.

genotyping: determination of the composition of all or part of the genome of a living organism.

glial cells: cells of the nervous system constituting the environment of the neurons. They ensure that it functions correctly by providing the neurons with mechanical support through the myelination of their axons (oligodendrocytes, Schwann cells), with nutrients and dioxygen (astrocytes), or with immune defense and waste elimination (microglial cells).

glutamatergic (neurons): neurons for which the neurotransmitter is glutamate, an ionized form of glutamic acid. This is the most common excitatory neurotransmitter in the central nervous system.

Green Fluorescent Protein (GFP): a naturally fluorescent protein. Under ultraviolet and blue light, it emits a green light. It can be associated with other proteins to form a fusion protein (for example with channelrhodopsin 2) and thus makes it possible to study them within cells.

grey (or gray) matter: part of the central nervous system consisting of the cell bodies of the neurons, their dendrites and the glial cells. It constitutes the cortex of the brain, the basal ganglia and the centre of the spinal cord.

gyrus: a ridge on the cerebral cortex bounded by sulci and giving the brain its external appearance. These gyri increase the surface of the cortex within a constant volume.

H

haemoglobin: a protein contained in the red blood cells (erythrocytes). It consists of four protein chains bound to an iron ion. Several types of molecules can bind to this latter: O_2, CO, CO_2, CN^-, H_2S. When dioxygen binds with iron, the haemoglobin is called oxyhaemoglobin (HbO_2). A haemoglobin molecule with no bound dioxygen is called deoxyhaemoglobin (Hb).

half-life (radioactive): the time after which one half of a population of radioactive atoms of the same type have spontaneously disintegrated according to a single process.

haplotype: a group of alleles situated in different *loci* of the same chromosome which are usually transmitted together. These alleles are genetically linked to one another.

hemodynamic (or haemodynamic) response: a physiological mechanism consisting in a local increase in blood flow in response to an increasing cell activity. In the case of the brain, this flow increases by about 5% in a few seconds when an area of the brain is activated. This mechanism is the basis of functional Magnetic Resonance Imaging (fMRI).

heterozygotic/homozygotic: an organism is heterozygotic for a gene when it possesses two different alleles of this gene on the same *locus* for each of its homologous chromosomes. It is homozygotic when it possesses two identical alleles.

higher functions (of the brain): the most sophisticated functions performed by the brain. These include the interpretation of messages from the senses, motricity, the production and understanding of language, memory or consciousness.

hormone: a chemical messenger secreted by an endocrine gland and carried by the blood or the lymph (biological fluid consisting of blood plasma and white blood cells). It acts remotely from its production site by binding with specific receptors.

I

image thresholding: computer processing of an image during which the value taken by each pixel undergoes binary modification (0 or 1) depending on whether it is below or above a fixed threshold value. This creates a contrasted image (black/white) from a grayscale image.

immunosuppression: the medical inhibition of the immune system. Immunosuppression is utilized in the case of an organ or cell graft, so that the immune system does not destroy the graft.

infrared (light): light invisible to the human eye, with a wavelength comprised between 780 **nm** and 1 mm. The **near infrared** is characterized by a wavelength ranging from 780 nm to 2,500 nm.

interfering RNA: single- or double-strand **RNA** whose interaction with a **messenger RNA** blocks the production of its associated **protein**. It can be used to study the expression of **genes** or the treatment of certain pathologies.

ischemia: a decrease in the supply of arterial blood to an organ.

isotope: all the atoms of a given chemical element have the same number of protons in their nucleus, but they can have different numbers of neutrons. In this case, these are said to be isotopes of this element. Some are stable, while others are unstable (**radioactive**).

L

lateralization (of the temporal lobe): certain **cognitive functions** performed by the temporal lobe (hearing, language, memory, complex shape analysis) are unequally distributed between the two hemispheres of the brain. Those which are more closely associated with language preferentially use the left lobe. They are said to be lateralized.

lentiviruses: a type of **viruses** of the **retrovirus** family, characterized by a lengthy period of incubation. These viruses can transfer a part of their **genome** to the host cell except during its division phase. They are excellent vectors for the transfer of **genes** (gene therapy) once their pathogenic power has been deactivated. HIV is a lentivirus.

ligand: in biology, an organic molecule liable to bind with a macromolecule such as a **protein**, modifying its chemical structure and its functionality.

light-emitting diode: a diode consists of the junction of two semiconductors. In one of them (type P), conduction is carried out by positively charged "holes". In the other one (type N), it is performed by electrons. When the diode is conducting (current flows from P to N), the mobile charges recombine at the junction. This is accompanied by emission of a photon. The characteristics of the diode can be adjusted so that these photons escape with a given wavelength: we then have a light-emitting diode.

lipids: substances in which the molecules consist of a hydrophobic part (not binding with the water molecules) and a hydrophilic head (capable of binding with water molecules). A lipid is said to be **cationic** when the hydrophilic head contains a positive charge, as is the case with an ammonium group.

localized proton NMR spectroscopy: a method for *in vivo* detection and chemical analysis comparable to **MRS** and limited to small regions, so as to be able to distinguish the weak signal emitted by the protons of the molecules studied.

locus*:** a precise location on a **chromosome**. A *locus* may or may not be occupied by a **gene**. Plural: ***loci.

M

magnetic moment: a property of sub-atomic particles (such as protons and electrons) associated with their **spin**. Modelled by a current loop, it is equal to the product of the current intensity by the surface of the loop and is represented by a vector perpendicular to this loop. It is expressed in $A \cdot m^2$.

Magnetic Resonance Spectroscopy (MRS): a chemical analysis method derived from the magnetic properties of atomic nuclei with a non-nil **spin**. Based on the principle of Magnetic Resonance Imaging (MRI), it allows to measure the frequencies emitted by the various nuclei of the atoms of a molecule. These frequencies are characteristic of these nuclei and of the chemical bonds set up between them. MRS offers the possibility of quantifying the **metabolites** present in the cells (**neurons** for example) and of calculating the kinetics of the reactions in which they are involved. MRS is also used in determining the structure of complex molecules such as **proteins**.

meta-analysis: a statistical method for combining the results from several independent studies on a given problem. Meta-analysis allows a more precise approach to the data by increasing the number of cases studied, thus enabling to draw more general conclusions.

metabolism: the set of chemical reactions which ensure the working of a living organism. Some of these reactions take place inside the cells of this organism and others outside (digestion for example).

metabolite: an organic compound resulting from the **metabolism**. This term is limited to compounds made up of small molecules.

methylation: substitution of a hydrogen atom by a methyl group ($-CH_3$) in an organic molecule. Certain **DNA** bases can be methylated. This is a process which modifies the expression of the **genes** comprising these bases.

micro: prefix μ for one millionth (10^{-6}). Refers to the **micrometric** scale which is that of the living cell. 1 **micrometre** (**μm**) = 10^{-6} m. Also called micron, it is one thousandth of a millimetre.

microglial cells: small **glial cells** related to macrophages and present in the **central nervous system**.

mitochondrial (deficit): an **enzymatic** deficit blocking the synthesis of Adenosine TriPhosphate (ATP) from the Adenosine DiPhosphate (ADP) contained in the **mitochondria**. In the case of dopaminergic **neurons**, the mitochondrial deficit disrupts the synthesis of **dopamine**.

mitochondrion: an organelle situated inside **eukaryotic** cells, whose main function is to store the energy from the glucose in Adenosine TriPhosphate (ATP) molecules and to release it on demand.

multicentric (study): refers to a scientific study conducted with volunteers from different medical centres. This enables to gather a large number of patients or healthy baseline subjects and obtain more precise medical data. If the study is performed in a single centre, it is said to be **monocentric**.

mutation (genetic): a transmissible alteration of the **genetic** message by modification of a **DNA nucleotide sequence**.

myelin: the **lipid** substance surrounding the **axons** thanks to the presence of oligodendrocytes (in the **central nervous system**) and Schwann cells (in the **peripheral nervous system**). Its presence increases the rate of propagation of the nerve impulses.

N

nano: prefix "n" for one billionth (10^{-9}). Refers to the **nanometric** scale which is that of atoms and molecules. 1 **nanometre** (**nm**) = 10^{-9} m. It is one millionth of a millimetre.

NEMS (Nano-Electro-Mechanical System): an electromechanical system of **nanometric** dimensions. These systems are used in emerging technologies (nanomotors, nanosensors, ultra-high-frequency oscillators, etc.).

nervous system (central and peripheral): the animal biological system responsible for the coordination of actions with the external environment and for rapid communication between the different parts of the body. It consists of **neurons**, **glial cells** and their interconnections. The **central nervous system** consists of the encephalon (brain, brainstem and cerebellum) and the spinal cord. The **peripheral nervous system** comprises the nerves and nerve ganglia.

neural (or neuronal) code: the way in which the **neuron** interaction between themselves and with the environment produces the brain's **higher functions**. This code is the result of the functional interaction of the neurons organized into numerous dynamic networks.

neural network: a mathematical model consisting of units schematically interconnected in a way similar to biological **neurons**. These models are the basis for artificial intelligence and enable to test certain functional hypotheses derived from neurophysiology.

neuroblast: a cell derived from a **neural** stem cell and progenitor of a **neuron**.

neurochemical (system): the set of **neurons** functioning with a given **neurotransmitter**.

neurofibrillary tangle: the normal working of the **axons** is ensured by microtubules the layout of which is governed by the tau **protein**. Neurofibrillary tangle results from a modification of the physicochemical properties of this protein, leading to its abnormal aggregation within the axons, to the formation of intracellular tangles and to major functional disorders. This neurofibrillary tangle is one of the two lesions characteristic of Alzheimer's disease.

neuromediator: see **neurotransmitter**.

neuron: a differentiated cell constituting the functional unit of the **nervous system**, ensuring the conduction of the nerve impulse, and the synthesis and transmission of **neuromediators**. Each neuron is formed of a cell body carrying numerous **dendrites** and prolonged by a single **axon**. The neurons are cells capable of conducting the nerve impulse using a centripetal process oriented from the dendrites to the cell body, and then a centrifugal process from the cell body to the axon. At birth, a human baby possesses about one hundred billion neurons.

neurotransmission (system): a set of **neurons** producing the same **neurotransmitter**. There are, for example, the **serotonergic**, **adrenergic**, **noradrenergic**, **cholinergic**, **glutamatergic**, dopaminergic and histaminergic systems.

neurotransmitter: a chemical substance synthesized by the **neurons** (and certain **glial cells**). It is contained in presynaptic vesicles and is released at a **synapse** when a nerve impulse arrives and diffuses to the postsynaptic receptors. The target neuron then takes the information, either to switch off or to switch on.

neurotrophic factors (or trophic factors of the nervous system): a family of **proteins** involved in the growth of **neurons** and in the upkeep of mature neurons.

noradrenaline: a **neurotransmitter** and **hormone** of the same family as **adrenaline** and **dopamine**. It acts on the effector organs and in numerous behaviours. It is a biological precursor of adrenaline.

noradrenergic (neurons): **neurons** for which the **neurotransmitter** is **noradrenaline**.

nucleic acid: a **polymer** consisting of a chain of **nucleotides**. There are two types: **DNA** (DeoxyriboNucleic Acid) and **RNAs** (RiboNucleic Acids).

nucleotide: an organic molecule consisting of a base, a sugar (pentose) and one to three phosphate groups. The bases can be *puric* (adenine or guanine) or *pyrimidic* (cytosine, thymine or uracile). **DNA** and **RNA** are made up of a chain of nucleotides. Adenosine TriPhosphate (ATP) and Adenosine DiPhosphate (ADP), which are involved in the storage and utilization of energy in the cells, are nucleotides.

O

Obsessive Compulsive Disorder (OCD): a mental disorder characterized by repetitive intrusive thoughts and/or repetitive and ritual behaviours aimed at reducing anxiety.

oncology: a medical discipline devoted to cancerous tumors.

oxidative stress: a type of aggression of the components of the cell due to reactive oxygen species and to reactive oxygen and nitrogen species.

P

parenchyma of the brain: all the **neurons** of the brain.

peptide: a short chain of **amino acids** linked by a **peptide bond**. Amino acids possess an acid function (–COOH) and an amine function. Two amino acids can thus bind together by attaching the carbon of the acid function from one of them to the nitrogen of the amine function of the other one (peptide bond).

phase I-II therapeutic test or clinical study: a study which assesses the lack of undesirable effects and tolerance of a treatment in healthy baseline subjects (phase I) and its therapeutic efficacy on patients (phase II).

phenotype: the set of apparent characters of a living organism resulting more or less directly from the expression of its **genome**. When used in a restricted sense, this term designates a particular character described by molecular, cellular or macroscopic criteria.

phoneme: the smallest unit of a spoken string that can be isolated and which allows to differentiate one word from another very similar word.

phonological (memory): the part of the working memory specializing in the processing of verbal and symbolic information. It is involved in reading, writing, oral comprehension and mental calculation.

piezoelectric (technologies): the piezoelectric effect is the property exhibited by certain materials of generating electrical charges when deformed (for example by compressing them) or conversely which deform (expansion or contraction in a certain direction) when an electric field is applied to them. This reversible process is exploited in the fabrication of micro-actuators such as micro-pumps.

polymers: substances in which the molecules are formed of the repetition of identical units called monomers: these are macromolecules.

polyomics: the grouping of several branches of molecular biology. They include genomics (study of the **genome**), transcriptomics (overall analysis of **genetic** expression), proteomics (study of proteomes, that is all the **proteins** in an organelle or an organism) or metabolomics (study of **metabolites**).

positron: the antiparticle of the electron. Their masses are identical, but their algebraic charges are opposed (in particular the electrical charge). Positrons can be emitted by certain unstable atomic nuclei through β^+ **radioactivity**.

pre-clinical (study or research): research on animal or cellular models to confirm the pertinence and safety of a diagnosis or treatment, before conducting trials on humans.

primary regions (of the brain): **cortical** zones coding sensorial information and controlling and executing movements. There are the primary **cortices** for hearing, sight, smell and taste, and the primary motor cortex.

primary visual cortex: a cortical area receiving and processing information from the retina. This area is situated on the inner face of the occipital lobe of each hemisphere of the brain.

prokaryote: a single-cell living organism with no nucleus. Its **DNA** is grouped in a zone called the nucleoid.

promoter sequence: a **sequence** of **nucleotides** close to the *locus* of a **gene** and essential for its transcription into **RNA**. The RNA polymerase binds to it before beginning RNA synthesis.

proteases (or peptidases): **enzymes** which break the **peptide bonds** of **proteins**.

protein: a biological macromolecule consisting of one or more chains of **amino acids** linked by **peptide bonds**. Proteins are synthesized in living cells from a **gene** by means of an **RNA** and the intervention of **enzymes**.

protoxin: a non-active precursor of a toxin.

Q

quantum: most of the physicochemical (and therefore biological) properties of matter are the result of the behaviour of its fundamental components (atomic nuclei and electrons), which is very different from what is visible at the macroscopic scale. Quantum mechanics describe matter at the atomic and sub-atomic scale. Numerous devices used in modern technology exploit some of the quantum properties of matter, such as the tunnel effect, the Josephson effect or **superconductivity**.

R

radioactive tracer (or radiopharmaceutical): a molecule with specific chemical properties (for example the receptor of a **neurotransmitter**) and one of whose atoms possesses a **radioactive** nucleus. Its location can then be detected by **Positron** Emission Tomography (PET) or by **Single Photon Emission Computed Tomography (SPECT)**.

radioactivity: the property exhibited by certain atomic nuclei to spontaneously transform into another one while emitting different types of radiation [α particles (nuclei of helium atoms), electrons (β^-), **positrons** (β^+), protons, neutrons] or by forming larger fragments (fission). The new nuclei (nucleus) produced are (is) generally obtained in an excited state and return(s) to a less excited state by emitting one or more **gamma photons**. This term also refers to all the radiation emitted by a substance containing radioactive atoms.

radio-ligand: a **ligand** containing a **radioactive** atom. Radioligands are used in **Positron** Emission Tomography (PET) and in **Single Photon Emission Computed Tomography (SPECT)**.

relaxation time weighting: in Magnetic Resonance Imaging (MRI), the **spins** of the protons (nuclei of hydrogen atoms) are first of all aligned with the direction of a constant field and then excited by an electromagnetic wave. When the excitation ceases, they resume their alignment and their magnetic properties return to nominal, with two different characteristic times (**relaxation time** T_1 or T_2) depending on whether it refers to the longitudinal or transverse component. The excitation and de-excitation times can be adjusted to highlight T_1 or T_2 separately. We then talk of **weighting** in T_1 or T_2. The images obtained in these two situations reveal different structures.

resolution: the separating capability of a detection device. It can be **spatial** (the smallest angular or linear separation between two objects, which in particular characterizes the ability of an optical system to differentiate or reproduce the details in a scene or its image) or **temporal** (the shortest time interval separating two successive instances of a signal over time and enabling them to be perceived as distinct).

retrovirus: a **virus** with a single strand of **RNA** possessing a viral **enzyme** (reverse transcriptase) capable of transcribing the RNA into **DNA**. This latter can then be integrated into the **genome** of the host cell.

RNA: RiboNucleic Acid. Consisting of puric (adenine, guanine) and pyrimidic (cytosine, uracile) **nucleotides**, this acid is involved in the expression of **DNA** information. In particular, the **messenger RNA**, a copy of a part of the DNA, specifies the **amino acid sequence** of a **protein** which it helps to synthesize. The **genetic** material of certain **viruses** consists of RNA.

S

sequence: order of the **nucleotides** in the biological **polymers** (**DNA**, **RNA**, **proteins**). **Sequencing** is the determination of this order.

serotonergic (neurons): **neurons** for which the **neurotransmitter** is serotonin. This one is for example involved in regulation of the circadian cycle (biological rhythm lasting 24 h).

Single Photon Emission Computed Tomography (SPECT): a medical imaging technique allowing to acquire planar images and 3D reconstructions of organs and their **metabolism** by means of gamma cameras rotating around the patient. These cameras detect the **gamma photons** emitted by a **radioactive tracer**, specific to the organ studied or to one of its functions, previously injected into the patient.

spin: an intrinsic property of any sub-atomic particle or any set of such particles. This **quantum** property, linked to magnetism, has no conventional equivalent but is of the same dimension as the orbital kinetic (or angular) momentum (product of a quantity of movement by the radius of the orbit). It is incorrectly defined as the rotation of the particle on itself. The spin of sub-atomic particles can take integer or half-integer value. That of an electron is equal to $\frac{1}{2}\,\hbar$ ($\hbar$ representing Planck's constant divided by $2\,\pi$).

superconducting (properties): properties of conducting materials which appear below a critical temperature. The conduction electrons then form pairs (known as Cooper pairs) and the resistivity of the material (ability to oppose the passage of an electric current) suddenly drops to a value close to zero. These properties are exploited in various devices such as Josephson

junctions, which are the basis of ultra-sensitive sensors capable of measuring very weak magnetic fields (SQUIDs).

superconductor: a material with **superconducting properties**.

synapse: an interaction zone between two **neurons** or between a neuron and another cell (muscle fibre for example). There are two types of synapses: electrical synapses which directly transmit the nerve impulse, and chemical synapses which allow the exchange of a **neurotransmitter**. The message transmitted can excite or inhibit the target cell.

T

terabyte: 10^{12} bytes or one thousand billion bytes.

theranostic (device): a device resulting from the combination of a diagnostic test and a targeted therapy, based on the result of this test.

tractography: a technique allowing 3D visualization of the **white matter** tracts by means of diffusion MRI.

transgene: a **gene** transferred from one living organism to another.

transgenic: refers to a living organism whose **genome** has been intentionally modified.

Turner's syndrome: a **chromosome** anomaly due to the absence of a sex chromosome: this is partial or total monosomy X0. This syndrome is characterized by several visible signs (short stature, swelling of the feet at birth, neck webbing), cardiac complications and growth disorders (impuberism). Hormone treatment is often necessary.

tyrosine: one of the 20 **amino acids**. Its molecule consists of a benzene ring carrying a hydroxyl group (–OH) and an amide in the "para" position, that is in the diametrically opposed position on the cycle. The **catecholamines** are synthesized from this amino acid.

U

ubiquitination: modification of a **protein** by the covalent bonding of one or more molecules of ubiquitin (itself a protein) with one or more of its lysines (one of the 20 **amino acids** constituting the proteins).

V

vectorization: the process whereby an active ingredient is introduced in the direction of a target (group of cells, organ, etc.) by means of a vector.

virus: an infectious agent that replicates only inside living host cells by using their machinery. It only contains a small **nucleic acid** (**DNA** or **RNA**).

Voxel-Based Morphometry (VBM): an analysis technique for images primarily obtained with Magnetic Resonance Imaging (MRI). The morphometric characteristics of a brain are usually determined by marking out the regions of interest on the image and directly calculating their volume. The VBM technique consists in deforming a 3D image of a subject so that it can be superimposed on that of a reference model (generally the mean of the representative subjects in the study), smoothing this image by averaging each voxel (three-dimensional pixel) with its immediate neighbours and then comparing image groups voxel-by-voxel in this reference space. It is thus possible to reveal singularities in the brains studied.

W

white matter: part of the **central nervous system** made up of nerve fibres which interconnect different **cortical** areas and transmit nerve impulses from one **neuron** to another. This matter consists of the **axon** extensions of the neurons organized into bundles of **myelinated** fibres.

Williams' syndrome: a syndrome due to the deletion (absence) of 1,500,000 base pairs from ***locus*** q11.23 of **chromosome** 7. It is characterized by malformation of the heart, mental retardation, developmental delay and an "elfin" facial appearance. The adult is rarely autonomous.

X

X-rays: an electromagnetic radiation with a wavelength ranging from 0.01 **nm** (10^{-11} m) to 10 nm (10^{-8} m). This range of wavelengths is between that of gamma rays and ultraviolet radiation. Discovered in 1895 by Wilhelm Conrad Röntgen, they pass through matter consisting of low mass atoms and can be used to create images showing certain internal structures of living organisms (skeleton in particular).

universcience présente

C3RV34U

L'EXPO NEUROLUDIQUE

Ⓜ Porte de la Villette / cite-sciences.fr

À PARTIR DU 16 SEPTEMBRE 2014

cité des sciences & de l'industrie

On en parle #Cerveau

Photo : Titus Lacoste - Réalisation papier : m.v.factory / figure.fr

france culture · france info · Le Parisien · L'EXPRESS · SCIENCE&VIE · Cerveau&Psycho · FUTURA SCIENCES · Syfy · SC discovery science

Une affiche sortie de la tête de BROCA & WERNICKE
L'agence partenaire de CERVEAU, l'expo neuroludique

www.cea.fr