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Imaging in Biomedical Research Mini-Symposium of the Division for Medicinal Chemistry (DMC) of the Swiss Chemical Society (SCS), at the Department of Chemistry, University of Basel, May 18, 2006

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Abstract: Imaging technologies have experienced rapid progress and are currently used widely both in medical diagnostics and in research. Imaging beyond X-ray and standard MRI became established in recent years. The extended set of imaging methodologies available allows methods to be selected according to the actual needs or even the combination of different imaging principles to obtain further improved read-outs. The symposium consisted of four overview lectures. Three speakers from academia and one speaker from industry described different techniques, recent developments and future needs from various perspectives in the lectures entitled: Non-Invasive Imaging in Biomedical Research: Annotating Structure with Molecular Information; Molecular Imaging with PET Tracers and Animal PET Scanners; Nuclear (PET- and SPECT-) Imaging Agents from Research to Approval (Perspectives from a Pharma Company Working on *in vivo* Diagnostics); Cognitive Neuroscience and Brain Imaging.

Keywords: Animal PET scanner · fMRI · Imagery · Molecular imaging · Neuroscience · PET-imaging agents · PET tracers · Recognition memory · SPECT-imaging · Visual perception

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Non-Invasive Imaging in Biomedical Research: Annotating Structure with Molecular Information Markus Rudin

In the Beginning There Was Structural Imaging

When around 1900 Wilhelm Röntgen developed a photographic plate showing the first X-ray image of his wife's hand he could not have dreamed of the success story biomedical imaging has undergone since. In the course of the last century essentially all 'windows to the body', *i.e.* spectral ranges for which absorption of radiation by tissue is low, have been exploited for imaging. Contrast in images arises from different properties of the tissue: scattering of X-rays at electrons for X-ray, magnetic properties of (hydrogen) nuclei for magnetic resonance imaging (MRI), or tissue specific compressibility values for ultrasound

imaging. These parameters depend on the tissue microstructure, and hence contain structural information. Consequently, these imaging techniques have been primarily used to study the normal and pathological anatomy.

Image quality is assessed by two criteria: Spatial resolution and image contrast. From a practical point of view, resolution is given by the smallest structure in the image that can be unambiguously identified. In the digital era, resolution is commonly given as the dimension of a picture element (pixel) or volume element (voxel). The ultimate limit of spatial resolution achievable depends on the intrinsic physical mechanisms underlying the imaging process. For instance, for MRI spatial information is inevitably lost due to diffusion of water molecules in the course of the measurement process, the mean free diffusion length being of the order of a few micrometers. In

practical terms, intrinsic limits are hardly reached for any imaging method and resolution is governed by instrumental factors and the inherent sensitivity of the imaging modality, which can be increased by spatial averaging thereby degrading resolution (Fig. 1). Contrast or rather the contrast-to-noise ratio (CNR) defines how well adjacent structures might be discriminated.

It was soon recognized that contrast can be altered by administration of suitable agents, molecules containing heavy elements (iodine) for X-ray, compounds with unpaired electrons for MRI. Alternatively, when administering compounds containing a metastable radionuclide, which decays upon emission of γ -quanta or positrons, and detecting emanating radiation using a suitable detection device (gamma camera, single photon emission computer tomography SPECT, positron emission tomography PET) the distribution of the radiolabels within the tissue can be reconstituted. Similar experiments can be carried out using fluorescent dyes. These contrast-enhanced or tracer based imaging approaches, besides enhancing contrast, offer a new range of applications: by measuring signal changes dynamically, *i.e.* as a function of time, they provide information on physiological and metabolic processes in a spatially resolved manner. Many of these functional readouts such as tissue blood flow, glucose metabolism, have become indispensable tools in radiology and nuclear medicine departments as sensitive indicators of tissue state.

Annotating Structural with Molecular Information

Classical imaging yields morphological and physiological information. These applications have largely defined its role as a diagnostic tool, pathologies being identified as deviation from normal anatomy or by altered physiological parameters. In addition to the detection and characterization of pathologies, the techniques can be used for monitoring disease progression, for stratification of patient groups, and for the evaluation of the response to therapeutic interventions.

Morphological and physiological aberrations, however, are the result of abnormal molecular processes and it is a reasonable hypothesis that quantitative mapping of these events *in vivo* might increase both the sensitivity and the specificity of diagnostic tools. In addition to structural and functional information, 'molecular' imaging methods provide readouts on levels of transcription and translation products, on critical molecules involved in signal transduction, and/or on protein-protein interactions (Fig. 2).

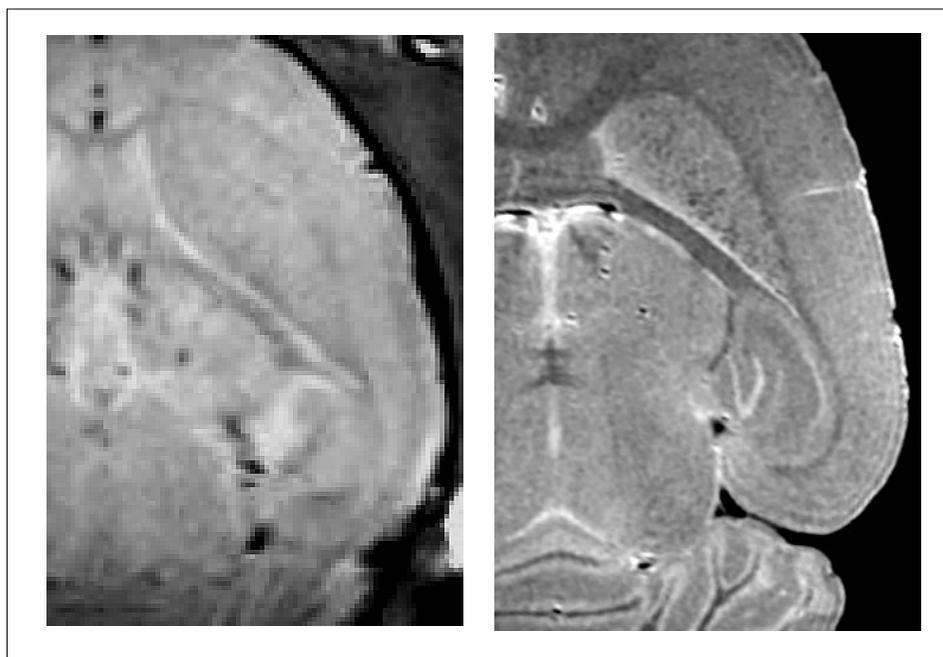


Fig. 1. High-resolution structural MRI of mouse brain *in vivo* (left) and of fixed brain specimen (right). Both data sets were collected with same MRI measurement protocol but different spatial resolution (voxel dimension = $100 \times 100 \times 300 \mu\text{m}^3$ and $60 \times 60 \times 60 \mu\text{m}^3$). Images were acquired in 12 min (*in vivo*) and 6 h (*ex vivo*), the higher resolution in the *ex vivo* image could only be achieved upon increasing the sensitivity by data averaging. For comparison, the ultimate resolution limit, the free diffusion path length is less than $1 \mu\text{m}$ for the experimental parameters used in the experiment.

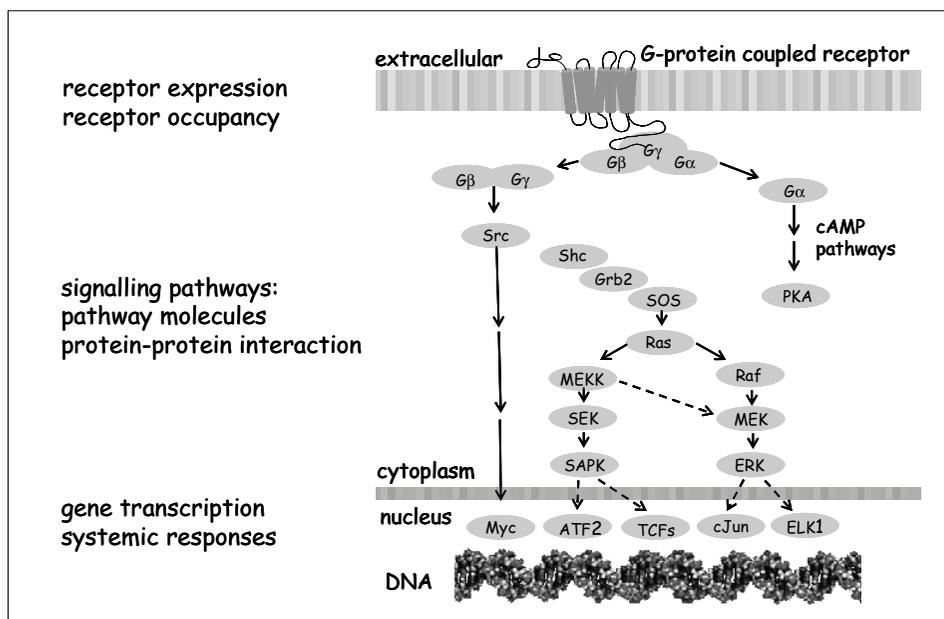


Fig. 2. Molecular imaging techniques provide information on receptor densities (gene expression) and receptor occupancy by a ligand/drug molecule, on the activation of signal transduction pathways by probing the expression levels of pathway molecules or by assessing protein-protein interactions, and on the functional consequences thereof such as the induction of down-stream gene expression or structural, physiological and metabolic responses.

The potential impact of such techniques on biomedical research will be significant, as diagnostic tools, for the identification of patient amenable to a specific therapeutic intervention, and for the identification and characterization of biomarkers with predictive quality with regard to clinical outcome (disease progression or therapeutic efficacy). Similarly basic biomedical research

will benefit from the quantitative assessment of a molecular process in the intact organism with all regulatory processes in place, enabling studies at the system level.

Spatial resolution and CNR define how well a specific structure can be identified. Yet the two quantities are inversely related: the better the spatial resolution the worse the CNR is. This is particularly relevant

with regard to molecular imaging applications. Tissue concentrations of critical biomolecules are low, typically sub-nanomolar, requiring the use of highly sensitive imaging modalities such as nuclear imaging, in particular SPECT and PET, and optical imaging. However, these techniques offer limited spatial resolution due to the underlying physical principles; in the case of PET due to the finite positron lifetime, errors in photon detection, and finite dimension of detector system, while in the case of optical imaging geometrical information is lost due to the diffusive propagation of light photons in tissue. Multimodal techniques, combining two or three complementary imaging modalities, allow some of these deficiencies to be overcome. The high spatial resolution provided by MRI or CT imaging can be combined with high sensitivity readouts derived from PET or optical imaging, thereby annotating anatomical structures with functional or molecular information. Moreover, multimodal imaging will allow the simultaneous recording of multiple parameters, *i.e.* tissue is characterized by a parameter profile instead of individual measures. This will enhance the specificity of the assay and provide unique mechanistic information.

Target-specific Assays

The key step in molecular imaging is the design of a target-specific probe that selectively provides cellular or molecular information. It is beyond the scope of this article to describe the various approaches in detail. The reader is referred to the literature [1–3]. Nevertheless, some basic design features and advantages/disadvantages of the respective approaches shall be briefly outlined.

The standard target-specific probe consists of a reporter moiety that is linked to a target-specific carrier molecule (Fig. 3). Reporter molecules can be paramagnetic complexes or super-paramagnetic nanoparticles for MRI, fluorescent dyes or quantum dots for optical imaging, or radionuclides for nuclear imaging (PET and SPECT). Target-specific moieties are low-molecular weight receptor ligands or enzyme substrates, antibodies and fragments thereof, or oligonucleotides.

Problems in designing target-specific probes are similar to those encountered in drug development. A molecular probe must have a high target affinity (in the nanomolar range), high target specificity, good bioavailability (membrane permeability) and favourable pharmacokinetic properties (the non-bound fraction should be rapidly eliminated to achieve a high target-to-background contrast), and a good safety profile. In addition, it should be detectable with

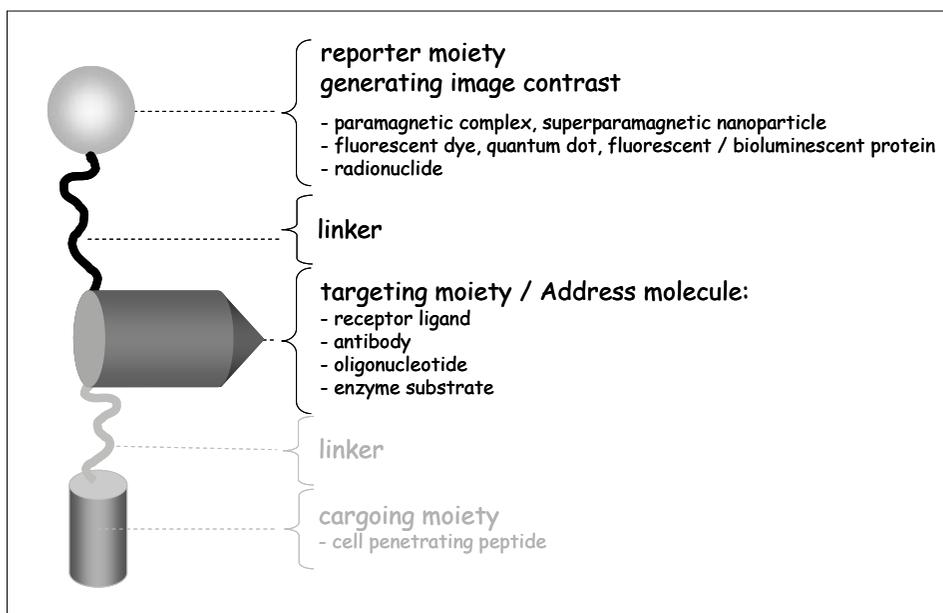


Fig. 3. Design of a target-specific probe combining as essential elements a signal-generating reporter with a targeting moiety. As additional feature, a cargoing element might be attached that enhances intra-cellular delivery of the construct.

high sensitivity at non-pharmacological doses; it should yield a signal when only a small fraction of targets is occupied. This might require specific signal amplification to be designed into the probe (see [1]). Obviously, the development of such probes, in particular for clinical use is difficult. Most molecular probes that are currently used in the clinics are based radio-labelling of known receptor ligands, thereby exploiting the inherent high sensitivity of the nuclear imaging methods.

Well-characterized examples are imaging ligands targeting somatostatin receptors (SSTRs). SSTRs are membrane-bound receptors belonging to the glycoprotein coupled receptor (GPCR) superfamily. Their endogenous ligand is somatostatin, a neuropeptide with a short plasma half life. SSTRs are highly expressed in neuroendocrine tumours and as such represent attractive targets for a tumour-specific imaging probe. A metabolically stabilized somatostatin analogue, octreotide, has been used as targeting moiety to which various reporter groups have been coupled (see Fig. 3). Administration of these ligands led to highly specific enrichment at the tumour site in patients suffering from neuro-endocrine tumours [4] and in animal models thereof [5]. An inherent problem when using such probes is the differentiation between signals arising from specifically bound (= target) from those of non-specifically bound or unbound reporter molecules (= background). The target-to-background ratio depends on the pharmacokinetic properties of the label and is a function of time; hence, optimal timing of the imaging experiment is essential.

The use of activatable probes alleviates this issue: high target-to background ratio is achieved by the fact that detection of the signal requires an interaction with the molecular target. Probe molecules that do not undergo activation by the target will remain ‘silent’. Activatable probes undergo structural modifications and thereby change their biophysical properties upon interaction with the target. Magnetic relaxivity and fluorescent parameters critically depend on the molecular structure. For example, coordination of (tissue) water molecules to the inner coordination-sphere of paramagnetic metal complexes (*e.g.* of Gd(III)) is essential for affecting the contrast in MR images by enhancing the relaxation of proton spins; critical factors determining relaxation efficiency are the number of free inner-sphere coordination sites, the water exchange rates, the overall molecular rotation (which depends on the molecular size). All these parameters can be influenced by a chemical reaction such as a cleavage of a molecular residue [6]. Similarly organic fluorophore properties depend on the molecular structure such as the length of the conjugated π -electron system. Intermolecular processes between fluorophores such as fluorescence quenching or fluorescence resonance energy transfer (FRET) depend on the intermolecular distance and hence, fluorescence intensity can be modulated by modulating this distance r ($\propto r^6$). Obviously radioisotopes cannot be used as reporter for activatable probes: nuclear decay occurs irrespective of the chemical environment of the radio-nuclide.

Examples of activatable probes are protease sensors [7]. They consist of a macromolecular biocompatible backbone (*e.g.* *m*-pegylated poly-L-lysine), to which a peptide motif, which is recognized and cleaved by the target protease, is coupled. Adjacent to the cleavage site two fluorescent reporters are attached; due to their proximity fluorescence is effectively quenched as long as the construct remains intact. Upon cleavage of the target peptide, the fluorophore groups are released, their intermolecular distance increases and a strong fluorescence is observed. The difference in fluorescence intensity between the bound and cleaved state are two to three orders of magnitude both *in vitro* and *in vivo*.

A third generic strategy is the development of reporter gene assays, which are widely used basic molecular biology tools that are now made available for *in vivo* imaging. Reporter proteins are expressed under the control of a target gene promoter sequence that regulates the expression of the target gene; hence, expression levels of the reporter gene product are used as a surrogate for the levels of the target protein. Different strategies for inserting the reporter gene may be applied: replacement of the target by the reporter gene leading to a functional knockout, expression as a target-reporter fusion protein using a peptidic linker, or co-expression of target and reporter gene. An interesting application of the use of reporter genes are assays probing protein–protein interaction such as the two-hybrid system [8][9], the protein fragment complementation assay [10] or protein splicing [11][12], which provide highly relevant information on the activation of signal transduction pathways. Typical reporter genes used for *in vivo* imaging are listed in Table 1.

Challenges in Biomedical (Molecular) Imaging

Imaging has matured to an indispensable technology in various fields of biomedical research and novel approaches providing molecular and cellular readouts are being developed at a rapid pace. Some of them will be translated into diagnostic clinical tools, while others are designed for fundamental research. In any case, a significant number of technical challenges have to be overcome.

Increase in Sensitivity

Imaging should enable full three-dimensional coverage of the target tissue with both high spatial and temporal resolution, as well as high sensitivity, *i.e.* high CNR or signal-to-background ratio. Yet, these requirements are mutually exclusive; increas-

Table 1. Reporter gene systems developed for *in vivo* imaging

Reporter gene product	Reporter ligand	Imaging modality	Reference
Herpes simplex virus-1 thymidine kinase (HSV1-tk)	[¹³¹ I] FIAU	SPECT	[13]
	[¹²⁴ I] FIAU	PET	[14]
β-galactosidase	EgadMe	MRI	[7]
Mutated transferrin receptor	MION	MRI	[15]
Firefly luciferase	D-luciferin	Bioluminescence	[16]
Fluorescent protein (GFP)	–	Fluorescence	[17][18]

FIAU: 2'-fluoro'-2'-deoxy-1-β-D-arabinofuranosyl-5-iodo-uracil; EgadMe: (1-(2-(β-galactopyranosyloxy)propyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane) gadolinium(III); MION: monocrystalline iron oxide nanoparticles

ing resolution will inherently compromise CNR. Hence, increasing sensitivity per unit time of current approaches as defined by

$$\frac{SNR}{time} \propto C \cdot \frac{S \cdot V_{voxel}}{\sigma_{noise}} \cdot \frac{1}{T_{acq}} N_{averages}^{1/2} \quad (1)$$

is essential. It can be increased by either increasing the terms in the numerator or by decreasing the terms in denominator. Various strategies are being pursued.

a) Increasing the signal

A trivial approach is to increase of the concentration *C* of the reporter moiety, *e.g.* by increasing the dose administered or by improving its pharmacokinetic properties. Limitations are dictated by potential biological interference of exogenous labels. More attractive is the development of marker molecules with increased signal output *S* per individual reporter entity. This is an area of active research in MRI and optical imaging. The efficiency of MR contrast agents increases when the relaxivity per paramagnetic centre is enhanced; similarly, fluorescence properties such as quantum yield or excitation/emission wavelength should be optimized to attain maximal signal intensity and minimal background interference due to tissue autofluorescence. A different avenue is specific for MRI: due to the low energies involved in MRI, only small population differences exist between ground and excited spin state at room temperature. Thus, only a small fraction of the nuclear spins (typically 10 ppm) contribute to the signal detected, *i.e.* MRI is inherently insensitive. Several orders of magnitude in sensitivity can be gained by increasing spin polarization, generating a so-called hyperpolarized state, an area of active research [19]. Amplification of the signal can also be achieved by increasing the number of the reporter groups associated to each targeting moiety, *e.g.*

attachment of *N* fluorescent groups to an antibody will lead to a *N*-fold increased fluorescence output assuming that the reporter groups behave independently, which is in general not the case. Finally, SNR per unit time can also be improved by decreasing the acquisition time *T_{acq}* per image allowing for a higher number of averages *N_{averages}* to be collected per unit time. A large number of fast sampling data strategies have been developed recently, in particular for MRI.

b) Decreasing noise

The noise figure σ_{noise} in the image is governed by two principal noise sources: noise generated by the sample to be imaged, $\sigma_{n,sample}$, and the noise generated by the detection device, $\sigma_{n,detector}$. Sample noise is intrinsically linked to the measurement principle and, therefore, unavoidable: reducing sample noise would also reduce the signal. Thermal noise is an important contributor to the detector noise figure; hence reducing the temperature of the detection devices can lead to significant noise reduction and correspondingly to increases in SNR/time. This has been realized both for optical imaging by using cooled charge-coupled devices (CCDs) or in MRI by using cooled radiofrequency receiver coils [20]. However, probe cooling will enhance SNR only in cases for which the variance of detector noise is at least comparable to that of sample noise.

Dealing with Complexity

Biological systems are inherently complex and simultaneous measurement of multiple parameters would be highly attractive. As different modalities provide complementary information, their combination might enable a comprehensive characterization of tissue, enhancing the diagnostic sensitivity and specificity. Today, the combination PET/CT linking functional/metabolic to anatomical information

has matured to a clinical standard. Further multiplexed techniques are currently under development. Hierarchical imaging, *i.e.* combining imaging across length scales will provide a biochemical/biophysical rationale for signal/contrast changes observed. For example, an MRI tissue parameter is given as the weighted average of the contributions of tissue constituents comprised in a voxel of typically $100 \times 100 \times 500 \mu\text{m}^3$. Combination with microscopy techniques might allow analysis of the micro-structure underlying the macroscopic observables. Complex biological processes might be analyzed at various levels. As an example, neuronal activation pattern might be analyzed as networks of interacting cerebral domains (whole brain level), excitation processing within a network composed of individual neurons (neuron cluster level), or at the sub-cellular level.

Quantification

Quantitative understanding of imaging readouts is critical in order to derive biomedically relevant information. This requires on the one hand the development or improvement of software tools for image registration, *i.e.* the exact alignment of images collected in different sessions or using different modalities, and segmentation, *i.e.* the identification of various tissue classes. Extracting biological information from imaging data sets is dependent on the basic understanding of the underlying physiological, metabolic or molecular processes. Sophisticated tissue models will help to rationalize imaging data. Multiplexed techniques will characterize tissue by parameter profiles (signatures) rather than individual parameters, which will be analyzed using statistical tools analogous to those used in *e.g.* metabonomics studies [21][22].

Imaging Agents

The key success factors in molecular imaging are probes/assays that provide target-specific information. Their development is closely related to drug-development (see above) and there is considerable synergy potential between the development of therapeutics and diagnostics.

Molecular imaging is currently still in its infancy; many of the applications to date are of the proof-of-principle type and the tools have not yet been developed to a stage where they can be used in a routine research or clinical setting. Consequently, many of the tools developed to date are far from being optimal; novel assays are required to tackle novel questions. It is, however, beyond doubt that imaging and in particular molecular imaging will make a significant impact on biomedical research.

Molecular Imaging with PET Tracers and Animal PET Scanners
Pius August Schubiger

Molecular Imaging and Positron Emission Tomography (PET)

Molecular imaging has become a very popular term in medicine. In the literature and at scientific meetings images are presented under the term ‘molecular’ –irrespective of the imaging method (CT, US, MRI, BLI or PET) and the information gained from the imaging method. However some methods will lead to *e.g.* structural images, whereas molecular imaging methods make molecular processes visible, quantifiable and traceable over time in a live animal or human [23]. Therefore I would define molecular imaging as ‘*in vivo* imaging of biological processes with appropriate specific molecular probes’.

So the real challenge in molecular imaging is the search for the ‘optimal’ molecular imaging probes followed by the search for the appropriate (imaging) method. Understanding biology at the molecular level needs molecules which are part of the biological processes underlying normal or diseased states. Imaging methods have been established using nuclear, optical and MRI imaging probes as explained by M. Rudin in the previous section. The choice of a certain imaging modality depends primarily on the specific question to be addressed. Answering those questions requires methods with specific properties on spatial resolution, sensitivity and specificity. The strength and weakness of various methods are discussed in many reviews (see *e.g.* [2][24]). In Table 2 some high-resolution small animal imaging systems are compared. It is obvious, that only PET has the sensitivity needed to visualize most interactions between physiological targets and ligands such as neurotransmitters and brain receptors. Therefore, if the question concerns monitoring drug distribution, pharmacokinetics and pharmacodynamics, PET is the only choice as a nuclear imaging technique for most organs.

Positron emission tomography is based on the annihilation of a positron. If a positron is emitted from a radionuclide it loses first its kinetic energy and then combines with an electron. The result of this process is annihilation of both particles through emission of two gamma rays. These gamma rays are emitted in opposite direction and can be detected coincidentally by PET detectors (see Fig. 4). Due to the ring geometry of the PET detectors and reconstruction computer programs it is possible to transform the registered gamma rays into a three dimensional representation of the radioactivity distribution. The main constituents of most biological molecules are carbon, nitrogen and oxygen. The incorporation of positron-emitting radionuclides such as carbon-11, nitrogen-13 or oxygen-15 with physical half-lives of 20, 10 and 2 minutes into such biomolecules leads then to optimal PET probes or PET radiopharmaceuticals because they are chemically indistinguishable from the non-labelled counterparts.

Development of New Molecular PET Probes

Once a desired PET molecular probe (PET ligand) is defined and synthesized, an *in vivo* animal test can successfully proceed only if a number of prerequisites have been fulfilled. These conditions for a PET ligand for the imaging of central nervous system (CNS) receptors are discussed below.

Selectivity/High Affinity for the Receptor (K_i or $K_d < 10^{-9}M$)

The equilibrium dissociation constant (K_d) of a drug receptor complex is the concentration of drug that occupies or binds to 50% of available receptor population. By definition, affinity is the reciprocal of the equilibrium dissociation constant and is ideally highest for the target site to be imaged. Considering that the concentration of binding sites (B_{max}) for most brain receptors is rather low (nano- to femtomoles per milligram tissue), PET ligands should have binding affinities in the subnanomolar

Table 2. Small animal imaging modalities

Imaging method	Spatial resolution	Temporal resolution	Sensitivity
Ultrasound	50 μm	>10 ms	10^{-3} Mol
CT	50 μm	>300 ms	10^{-3} Mol
MRI	100 μm	>50 ms	10^{-5} Mol
MRS	1 mm (?)	>60 ms	10^{-5} Mol
BLI	1–3 mm (depth!)	1 ms	
PET	>1 mm	>1 s	10^{-9} – 10^{-12} Mol

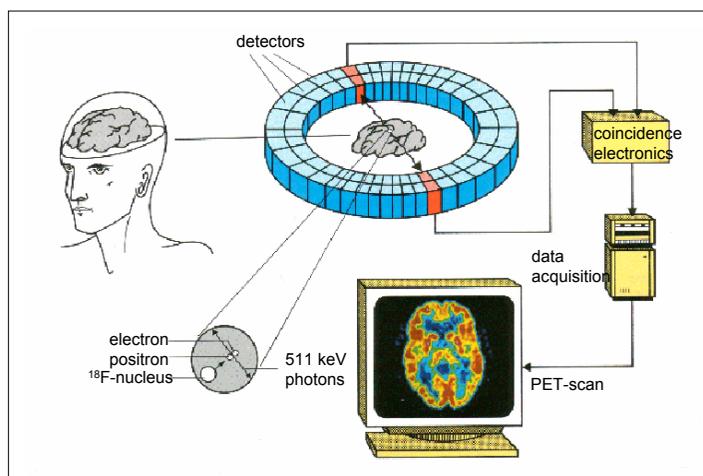


Fig. 4. The principle of PET

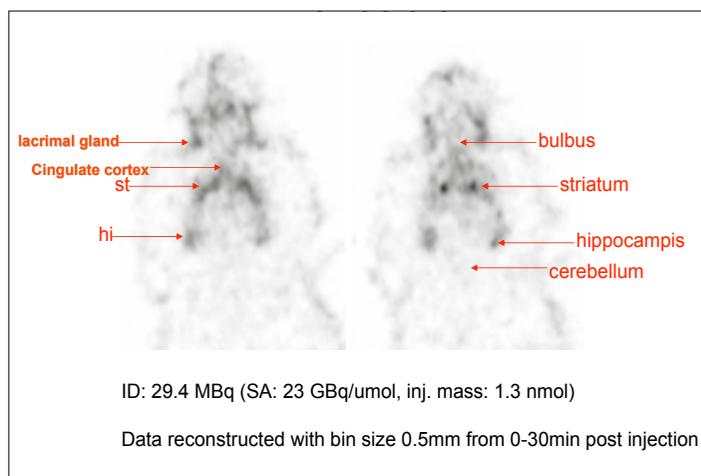


Fig. 5. PET studies of $[^{11}\text{C}]\text{-ABP688}$ uptake in a rat brain

range. But too high an affinity can render a PET radiopharmaceutical useless because its uptake may become blood flow dependent instead of being dependent of the rate of binding.

Specific Radioactivity

Specific radioactivity refers to the amount of radioactivity per unit mass of a radiopharmaceutical. Unlike radioligands used only for *in vitro* binding assays, radioligands used for *in vivo* brain receptor imaging must be prepared in high specific radioactivities so that only a small percentage of the total number of available binding sites is occupied by the radioligand. Low specific radioactivity may lead to saturation of the binding sites which may result in pharmacological effects or toxicity.

Metabolism and Position of Label

Since PET cannot discriminate between signals from parent radioligand and radiolabelled metabolites, it is essential that PET ligands do not undergo rapid metabolism over the period of PET measurements. It has therefore to be verified that radioactive metabolites that are formed in the course of data acquisition do not contaminate the PET signals. The position of the radiolabel in a molecule is also another crucial factor and has to be considered very carefully during the planning of the synthetic approach since the loss of the radiolabel in a molecule by metabolic degradation will limit its usefulness as a PET ligand.

Blood-Brain Barrier Permeability

For a CNS radiopharmaceutical to be useful as an imaging agent, it must be lipid soluble and should readily pass the blood-brain barrier (BBB). The octanol/water partition coefficient, *P*, is often used

as a predictor for BBB penetration. The *P* values can be computed or experimentally measured and log *P* values between 2 and 3 are generally considered optimal. The criterion necessarily holds for diffusion-mediated transport systems but not for specialized active transport systems as it is the case for amino acids.

Clearance Rate and Binding to Proteins

Also of importance are rapid clearance rates from blood and non-specific binding sites. A low binding of radioligand to plasma proteins is essential since only the free fraction of radioligand in plasma is available for diffusion out of the vascular space.

A first example concerns potential PET-ligands for the metabotropic glutamatergic receptor subtype 5 (mGluR5). Table 3 shows the structures of potential PET ligands with their respective binding affinities and lipophilicity values. This is a selection of some of the ligands we recently synthesized and evaluated [25][26].

The only compound which is selective and shows high affinity is $[^{11}\text{C}]\text{-ABP688}$. It is the first known PET-ligand displaying an *in vivo* distribution pattern consistent with the known regional density of mGluR5. Fig. 5 shows an animal PET study in a rat brain. The results indicate that this compound has the potential to become a valuable tracer for imaging the mGluR5 distribution in humans using PET. Furthermore it could be of great value for the selection of appropriate doses of clinically relevant candidate drugs that bind to the mGluR5.

The second example is about the uptake of $[^{18}\text{F}]\text{-FECNT}$ (2 β -carbomethoxy-3 β -/4-chlorophenyl)-8-(2-fluoroethyl)-nortropine, a dopamine-transporter ligand in the striatum of mice. Parkinson's disease (PD) is characterized by a progressive degeneration of nigrostriatal neurons and depletion of dopamine in the striatum. This striatal degeneration can be analyzed non-invasively by small animal PET imaging using the DAT tracer $[^{18}\text{F}]\text{-FECNT}$ in a mouse model of PD. Furthermore, such an experimental

Table 3. Development of a molecular PET-probe: structures of some mGluR5 PET ligands

Ligand	Structure	MW (g/mol)	LogP _{calc} (LogD _{exp})	KD (nM)	IC ₅₀ (nM)
MPEP 6-methyl-2-(phenylethynyl)-pyridine		193.24	3.77		34
M-MPEP 2-methyl-6-(methoxyphenyl) ethynyl-pyridine		223.27	3.64	3.4	
M-FPEP 2-methyl-6-(3-fluoro phenylethynyl) pyridine		211.23	3.93 (2.7)	1.4 + 1.1	9
ABP688 3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone O-methyl-oxime		239.31	2.44 (2.4)	1.7 + 0.2	3

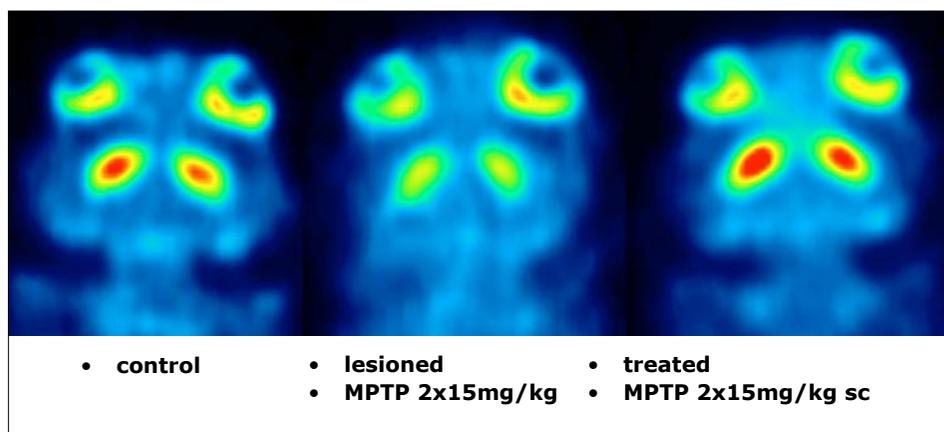


Fig. 6. [¹⁸F]-FECNT PET in healthy, lesioned and treated animals

approach can be applied to develop novel drugs against PD.

Fig. 6 shows representative coronal slices of the head of three different mice injected with the [¹⁸F]-FECNT: a control mouse (left), a lesioned mouse (middle) and a lesioned mouse that was treated with a drug (right).

Animals were lesioned with MPTP (2 × 15 mg/kg) and received the drug in the drinking water. All PET images were obtained by adding data from 1–31 min post injection and are normalized to the injected dose per body weight. Drug treatment leads to a clear recovery of [¹⁸F]-FECNT uptake in the striatum of MPTP-lesioned mice, thus demonstrating the drug's efficacy to restore dopaminergic neurotransmission.

Nuclear (PET- and SPECT-) Imaging Agents from Research to Approval (Perspectives from a Pharma Company Working on *in vivo* Diagnostics)

Matthias Bräutigam

What was celebrated as a sensation over 100 years ago with the discovery of the X-ray has since developed into a highly specialized field of medicine: diagnostic imaging. It allows physicians to diagnose diseases at an early stage and thus begin a suitable treatment. Nowadays, additional diagnostic procedures such as computer tomography (CT), magnetic resonance imaging (MRI), ultrasound and nuclear diagnostic methods are available in addition to the classic X-ray.

Schering has been a pioneer in the development of contrast media for these technologies. Today, Schering's research focuses on new and innovative approaches in

the fields of magnetic resonance imaging, computer tomography, optical and molecular imaging.

Molecular imaging technologies based on radioactive isotope techniques such as PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) gain more and more relevance for the *in vivo* characterization of biological processes on the cellular and molecular level. They bear the potential for an earlier and more specific diagnosis (*e.g.* staging), an earlier monitoring of therapeutic interventions and a monitoring of a 'broader' and 'more specific' set of pathophysiological markers (*e.g.* glucose metabolism, proliferation, apoptosis, expression of erb B2 receptor, expression of estrogen receptor, specific distribution of [¹⁸F]-herceptin and [¹⁸F]-fluoroestradiol).

FDG ([¹⁸F]-fluoro-deoxyglucose) represents an example for a clinically available nuclear PET imaging reagent. Due to the limited half live of the [¹⁸F]-isotope (2 h), very challenging technological prob-

lems had to be solved to obtain finally the approval for its clinical use: ultra rapid production of [¹⁸F]-fluoro-deoxyglucose including isotope production in a cyclotron, most rapid chemical synthesis, purification, analytics and QA release (Fig. 7). The fact that only micro-doses of labelled tracer have to be applied clinically facilitates regulatory approval; *e.g.* <100 µg (0.08 µg for FDG-PET) is injected (<1/100th of the dose calculated to yield a pharmacological effect). FDG is currently approved for diagnostics in a broad set of different cancer types and for Alzheimer's disease.

¹²³I labelled scFv(L19), a recombinant human antibody fragment with sub-nanomolar affinity for the Extra-Domain B (EDB) of Fibronectin is an example of a clinically used nuclear SPECT compound (Fig. 8). It serves as marker of angiogenesis, the growth of new blood vessels in tumour tissue. ED-B expression is relevant in primary tumours of various histotypes, thus the Extra-Domain B (EDB) Fibronectin marker can be used successfully in clear cell carcinoma of the kidney, invasive ductal carcinoma and invasive lobular carcinoma associated with breast cancer, in adenocarcinoma associated with prostate cancer and in oral squamous cell carcinoma.

The cost of commercialising a new agent for diagnostic imaging is substantially lower than that for a new (cold) therapeutic drug (in the range of \$100–200 million) with a total development time of 8–10 y. A blockbuster agent (most of which have been on the market for some time) has peak sales of \$200–400 million. Obviously, it is more attractive to develop an imaging agent for a wide range of applications, the development of an agent for a rare disease is possible only if high selling prices can be achieved.

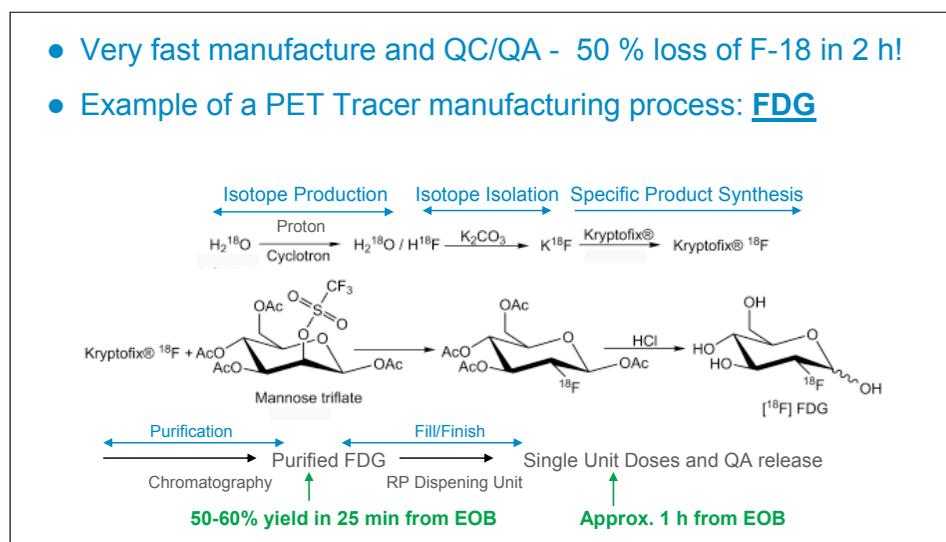


Fig. 7. PET tracer production

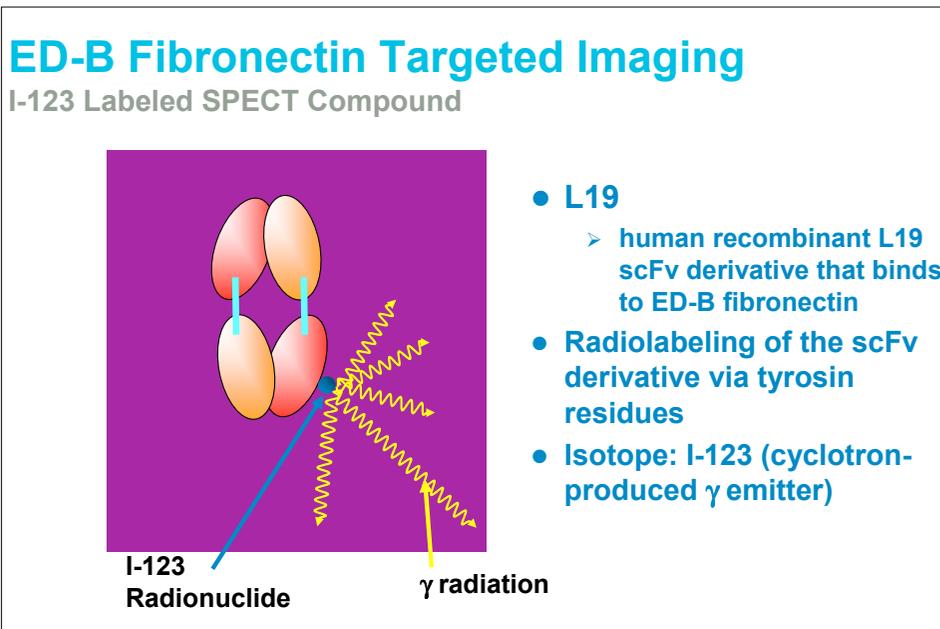


Fig. 8.

In the future, we expect a more close collaboration between pharma and diagnostics divisions and imaging agents will not only be used as diagnostic agents to detect and monitor specific diseases but also as markers to speed up the development of therapeutic drugs and to manage patient care post approval.

Cognitive Neuroscience and Brain Imaging

Hanns Möhler and Alomit Ishai

Introduction

Cognitive neuroscience is the attempt to delineate the neural substrates which correlate with higher cognitive brain functions such as language, emotions, memory or consciousness. Complex brain functions involve the operation of multiple brain ar-

reas encompassing distributed neuronal networks which act in concert. The distributed architecture of the brain can be visualized by functional brain imaging technologies. As an example, functional magnetic resonance imaging (fMRI) studies of visual perception, imagery and memory are outlined below. In the future, however, new methods will be needed to monitor the functional connectivity between brain areas to gain further insights into the operation of the human brain.

What is Cognitive Neuroscience?

In 1895, a little-known Viennese neuropsychiatrist named Sigmund Freud, wrote a work entitles 'A project for a scientific psychology' in which he proposed that the cognitive mechanisms of normal and abnormal mental phenomena could be explained through orderly and rigorous study of brain systems. The empirical basis for this endeavour was, however, very small. Although the 'Neuron-doctrin' had recently been published, it was not known how neurons interact; neither the synapse nor the electrophysiological properties of nerve cells were known. Freud abandoned both the project and neuropsychiatry [27]. This example illustrates the difficulty in relating mental phenomena to a neuronal substrate at the time, due to the lack of a conceptual and methodological framework. In the subsequent 50 years, psychology and

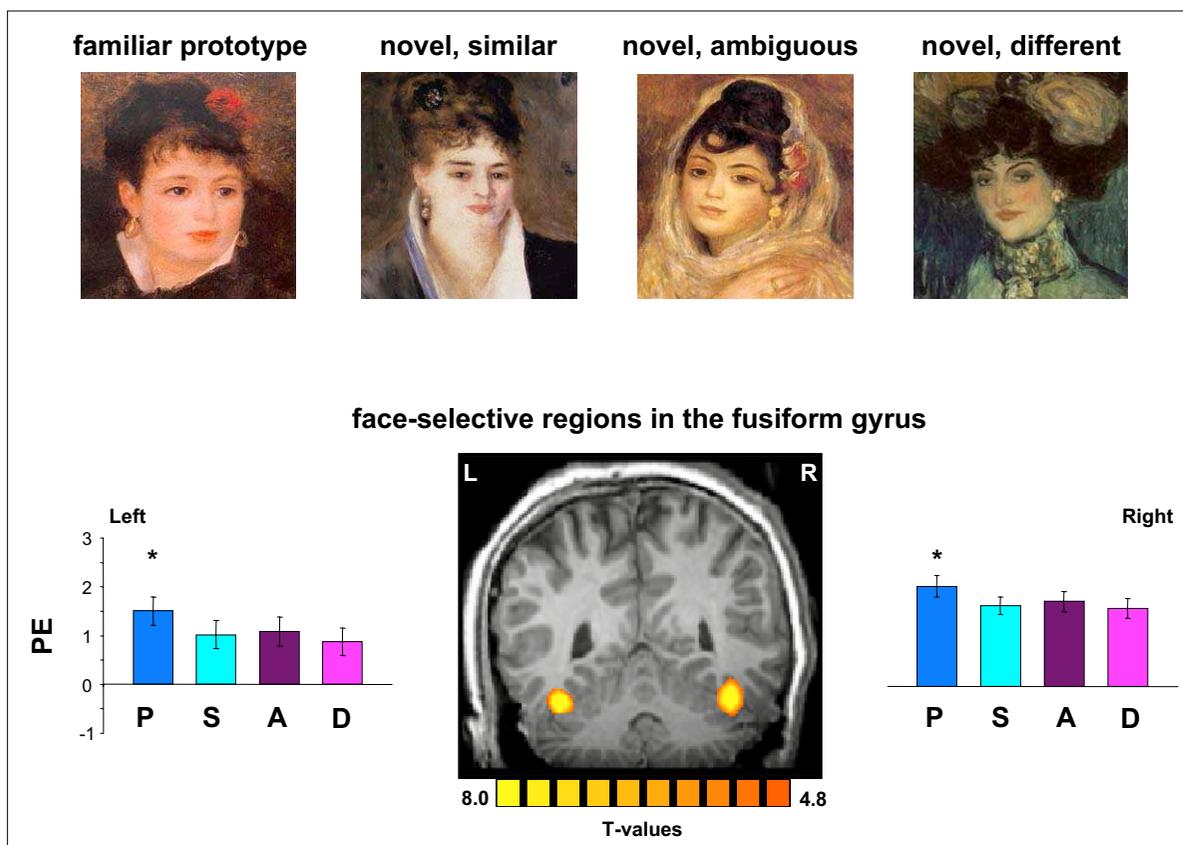


Fig. 9. Subjects memorized prototypes of portraits by Renoir. Four days later, a memory retrieval session was conducted in the MR scanner. The familiar prototypes (P) were mixed with new exemplars that were visually similar (S), ambiguous (A) or different (D), and subjects indicated whether they had seen each picture before. The fMRI data analysis revealed activation in the lateral fusiform gyrus, a face-responsive region. Within this region, familiar prototypes evoked stronger activation than all the new exemplars, regardless of their visual similarity [29].

neuroscience went their own separate ways. In various schools of psychology, behaviour was studied purely as an input–output pattern of observable aspects. In this view the brain itself was simply considered as a black box. The operation of the brain was not taken into account, as if the process by which information is transformed in the brain cannot influence behaviour. On the other hand, the discipline of neurobiology focused on the efforts to understand the structure and function of neural circuits, in particular with regard to sensory and motor systems based on the conceptual framework of neuronal signalling of individual cells and networks. With the advent of molecular biology, advanced morphology and techniques for studying the activity of single cells, the first correlations between cognitive processes and the pattern of neuronal firing in specific brain areas were found.

In the seventies and eighties, cognitive psychology acknowledged that internal representations are an essential component of behaviour. It was recognized that without direct access to the neural substrates of internal representations it appeared difficult, if not impossible, to understand the path from perception to action. As a consequence, the way behaviour was studied changed both in experimental animals and in humans. The focus had now shifted to the question of information processing in the brain that mediates behaviour.

The Distributed Architecture of the Brain

Today, the two lines of research, cognitive psychology and neurobiology, have merged into a new discipline termed cognitive neuroscience. In the frame-work of cognitive neuroscience, a cooperation takes place between psychology, neuropsychology, psychiatry, neurology, systems physiology, cell biology and molecular biology. This interdisciplinary approach enables the scientific study of memory, perception, action, language, emotion and conscious awareness.

In this setting, imaging technologies (see M. Rudin and A. Schubiger) take centre stage in the cognitive neurosciences. Functional MRI is a technique that permits the localization of regions in the human brain that are activated during cognition, with fine spatial and temporal resolutions (millimetres and seconds, respectively). Due to its non-invasive nature, fMRI is particularly useful for studying the neural basis of higher cognitive phenomena, such as visual imagery, which are not easily simulated in animal models (see below). Modern imaging technologies are therefore a great step forward in explaining behavioural, men-

tal and psychological phenomena through processes of concerted local brain activities. A distributed functional architecture of the brain became apparent.

The Representation of Visual Memory

Most if not all brain functions involve various brain areas acting in concert. A visual memory task is outlined below to illustrate the distributed architecture of the brain. Recognition memory depends on the visual similarity between a known prototype and a novel exemplar. For instance, the paintings of Picasso's blue period share certain characteristics which permit us to decide whether a new picture which is presented to us belongs to this period or not. To analyse how recognition memory is represented in the brain, a functional magnetic resonance imaging (fMRI) study was recently performed [28]. In an encoding session, subjects were instructed to memorize paintings (portraits by Modigliani and Renoir, landscapes by Pissarro and Van Gogh, abstract paintings by Kandinsky and Mirò) and were explicitly told that the prototypes from each painter belong to a category of paintings with a unique style (Fig. 9). Each picture was presented on the centre of a computer screen for 5 sec and was repeated four times to enable deep encoding. Four days later, the subjects performed a memory retrieval task in the MR scanner. The familiar prototypes from each painter were randomly presented among various new exemplars. The new pictures were either visually similar to the familiar prototypes, ambiguous, or dissimilar (Fig. 9). Each picture was presented for 3 sec and the subjects pressed a button to indicate whether they had seen the picture before ('yes' for the prototypes, 'no' for the new exemplars). The fMRI results revealed activation within a distributed cortical network that included visual, limbic, parietal and prefrontal regions.

Visual Brain Areas

Perception of portraits, landscapes and abstract paintings evoked activation in face- and object-selective regions in the visual cortex. In the lateral fusiform gyrus, a face-responsive region, portraits elicited stronger activation than landscapes and abstract paintings. In the medial fusiform and para-hippocampal gyri, regions that respond to houses and places, respectively, landscapes evoked stronger activation than portraits and abstract paintings. In the posterior fusiform gyrus, abstract paintings evoked stronger activation than portraits and landscapes. Within these face- and object-selective regions, familiar prototypes of portraits and abstract paintings evoked

stronger responses than the new exemplars (Fig. 9). Taken together, the patterns of activation observed in the visual cortex indicate that explicit encoding of categorical paintings results in stimulus-specific representations.

Attention-related Brain Areas

Activation in attention-related regions in parietal cortex, namely the intraparietal sulcus and the superior parietal lobule, revealed stronger responses to the familiar prototypes than to the new exemplars of portraits, landscapes and abstract paintings. Moreover, activation within these regions was reduced with decreased similarity between the new exemplars and the prototypes. The enhanced activation evoked by the prototypes and the reduced activity elicited by the visually different exemplar suggest that the attention-related regions process the segmentation of old from new items.

Memory-related Brain Areas

Recognition memory of prototypes of paintings revealed activation in multiple memory-related areas with distinct patterns of response: in the caudate, insula, and anterior cingulate cortex, the familiar prototypes elicited stronger activation than the new items, whereas in the precuneus, superior temporal and superior frontal gyri the new, visually different exemplars evoked stronger activation. Finally, in the hippocampus, the similar items evoked weaker activation than the other novel exemplars. In summary, the results show that recognition memory is mediated by a distributed cortical network where activation is modulated by the visual similarity between familiar prototypes and novel exemplars. Face- and object-selective regions in the visual system store stimulus-specific representations, attention-related regions in parietal cortex detect the familiar prototypes and memory-related areas classify novel exemplars as a match or a mismatch.

Visual Perception and Visual Imagery

Complex pictorial information can be represented and retrieved from memory as mental visual images. Functional brain imaging studies have shown that visual perception and visual imagery share common neural substrates. The type of memory (short- or long-term) that mediates the generation of mental images and the effects of focal attention to features of visual mental images were addressed in another fMRI study [29]. Famous faces were used to localize the visual response during perception and to compare the responses during visual

imagery generated from short-term memory (subjects memorized specific pictures of celebrities before the imagery task) and imagery from long-term memory (subjects imagined famous faces without seeing specific pictures during the experimental session). Visual perception of famous faces activated face-selective regions in the inferior occipital gyrus, lateral fusiform gyrus, superior temporal sulcus, and the amygdala. Although the pictures were grey-scale photographs of contemporary Hollywood celebrities with neutral expressions and the task was passive viewing, bilateral activation in the amygdala was observed in all subjects, probably due to the affective component of viewing good-looking faces. Interestingly, small subsets of these face-selective regions were activated during visual imagery. These results suggest that sensory representations of faces stored in face-selective regions in the visual cortex and the amygdala are reactivated during the generation of visual images. The view that the neurons that 'see' are also the neurons that 'remember' is supported by early evidence that sensory regions in the temporal lobe are associated with memory retrieval [30].

Visual Imagery, Memory, and Attention

Visual imagery of famous faces activated a network of regions composed of bilateral calcarine, hippocampus, precuneus, intraparietal sulcus, and the inferior frontal gyrus. Within these regions, imagery generated from short-term memory evoked more activation than imagery from long-term memory. Thus, when subjects were presented with specific pictures of celebrities and memorized them prior to the imagery task, the brain activity during imagery was stronger, suggesting that maintaining a trace in a 'working memory buffer' for several seconds likely mediates the generation of visual images. Regardless of memory type, focusing attention on features of the imagined faces (e.g. eyes, lips, or nose) resulted in increased activation in the right intraparietal sulcus and right inferior frontal gyrus. These results suggest differential effects of memory and attention during the generation and maintenance of mental images of faces.

Taken collectively, these findings revealed that visual imagery of famous faces may be implemented by content-related activation in small subsets of visual cortex, and 'top-down' mechanisms in parietal and frontal cortex that mediate the retrieval of face and object representations from long-term memory and their maintenance through visual imagery.

Outlook

Imaging technologies permit the identification of areas of neuronal activity. In the future, methods will have to be developed to visualize the flow of information between brain areas in governing behaviour. The connectivity between neural networks will have to be monitored in order to understand how the different neuronal networks interact in the distributed architecture of the brain. Knowledge on the task-dependent dynamics of the operational connections between brain areas will be needed.

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