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PO Box 117
221 00 Lund
+46 46-222 00 00

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Imaging in Cell Based Therapy for Neurodegenerative Diseases

Deniz Kirik[1,*], Nathalie Breysse[1], Tomas Björklund[1], Laurent Besret[2], and Philippe Hantraye[2]

[1] Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, BMC A11, 22184, Lund, Sweden

[2] URA CEA CNRS 2210 Isotopic Imaging UIIBP Unit and Program Imagene, Service Hospitalier Frédéric Joliot, 91401 Orsay, France

*: corresponding author

Contact address for CA: Wallenberg Neuroscience Center
Department of Experimental Medical Science
Section for Neuroscience
Disease Modeling Group
Lund University
BMC A11, 22184, Lund, Sweden
Tel: +46-46-2220564; Fax: +46-46-2223436;
e-mail: deniz.kirik@med.lu.se

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Abbreviations: AADC: aromatic L-amino acid decarboxylase; AD: Alzheimer disease; ADC: apparent diffusion coefficient; BOLD: blood oxygen level dependent; CIT: 2-β-carbomethoxy-3-β-(4-iodophenyl)tropane; CNTF: ciliary neurotrophic factor; COMT: catechol-O-methyl-transferase; Cr: creatine; DA: dopamine; DAT: dopamine transporter; DOPAC: 3,4-dihydroxyphenylaceticacid; DWI: diffusion weighted imaging; DTI: diffusion tensor imaging; DTBZ: dihydrotetrabenazine; FDG: fluorodeoxyglucose; FMRI: functional magnetic resonance imaging; GABA: γ-aminobutyric acid; GAPDH: glyceraldehyde phosphate deshydrogenase; Glx: glutamine/glutamate; Gd: Gadolinium; HAP: Huntington associated protein; HD: Huntington disease; HVA: homovanillic acid; L-DOPA: 3,4 dihydroxyphenylalanine: MAO: monoamine oxidase; MRI: magnetic resonance imaging; MRS: magnetic resonance spectroscopy; MSA-P: striato-nigral variant of multiple system atrophy; NAA: N-acetylaspartate; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; NMDA: N-methyl-D-aspartate; NMR: nuclear magnetic resonance; NOS: nitric oxide synthase; PCr: phosphocreatine; PD: Parkinson disease; PE2I: *N*-(3-iodoprop-(2E)-enyl)-2β-carboxymethoxy-3β-(4'-methylphenyl)nortropane; PET: positron emission tomography; PIB: Pittsburg compound-B; SN: substantia nigra; SNc: substancia nigra pars compacta; TH: tyrosine hydroxylase; VMAT: vesicular monoamine transporter; VTA: ventral tegmental area

Abstract

Fetal cell transplantation for the treatment of Parkinson's and Huntington's diseases have been developed over the last two decades and is now in early clinical testing phase. Direct assessment of the graft's survival, integration to the host brain, and its impact on neuronal functions requires advanced *in vivo* neuroimaging techniques. Due to its high sensitivity, positron emission tomography is today the most widely used tool to evaluate the viability and function of the transplanted tissue in the brain. Nuclear magnetic resonance techniques are opening new possibilities for imaging neurochemical events in the brain. The ultimate goal will be to use the combination of multiple imaging modalities for complete functional monitoring of the repair processes in the central nervous system.

Parkinson's disease

Parkinson's disease (PD), first described in 1817 by James Parkinson in a monograph headed "an assay on the Shaking Palsy", is today recognized as the second most common neurodegenerative disorder, with a prevalence of 0.1% of the global population. Interestingly, although PD is an age-related disorder afflicting about 1% of people over 65 years old and 4-5% of people over 85, 5-10% of the patients are under 40, supporting the fact that PD is considered as a major social health problem with significant economic impact [1, 2]. Since PD exists as both an idiopathic and familial disorder, controversies still persist on the respective contribution of environmental versus genetic factors in the genesis of the disease. Clues on the genetic component have mainly been introduced by identification of mutations or overexpression in genes encoding for various proteins including parkin, α-synuclein, ubiquitin carboxy hydrolase L1, PINK1 and DJ-1, in familial cases of PD [3, 4]. Environmental factors such as organic insecticides, herbicides, fungicides or exposure to metals have also been identified as potential risk factors in PD [5, 6]. It seems however that both familial and sporadic PD, lead to a convergent pathogenic pathway involving identical and multifactorial players identified as mitochondrial dysfunction, oxidative stress and protein aggregation, responsible for the damage and loss of dopamine (DA) neurons.

Clinical features

The clinical characteristics of PD include both motor and non-motor manifestations. While the reader is referred to several comprehensive reviews on this topic (see refs. [7-10]), here we will give only a brief description of the clinical findings. The cardinal motor features of PD are resting tremor, rigidity, akinesia/bradykinesia and postural instability/gait disturbance, which usually develop over many years as the disease progresses. Tremor, rigidity and akinesia appear as the first components of the symptomatic profile. These symptoms are usually expressed asymmetrically, primarily affecting one segment of the body or a hemibody. Hypomimia and eye blinking, as well as hypophonia are also early manifestations of the disease. Three to five years after diagnosis, symptoms spread to the controlateral limbs and axial musculature. Postural instability and gait disturbance occur at later stage of the disease and lead to loss of equilibrium and falling. PD diagnosis is established when at least two motor symptoms are found, at least one being tremor or bradykinesia, and when patients respond positively to 3,4-dihydroxyphenylalanine (L-

DOPA) therapy [11]. Apart from the clinical motor hallmarks, subtle cognitive dysfunctions, abnormalities in autonomic functions as well as psychiatric disturbances such as anxiety and depression are also reported (see [7, 12-14] for review).

Neuropathological Findings

The phenomenon underlying the occurrence and development of the symptomatology for the vast majority of PD patients remains unknown. In 1895, Brissaud suggested that a neuronal loss at the level of the substantia nigra, a nucleus located in the ventral midbrain that contains high amounts of neuromelanin pigment, might be a key factor for such manifestations [15]. About 10 years later, Lewy described intraneuronal inclusions in the dorsal motor nucleus and the nuclei of Meynert [16], and Tretiàkoff reported both a severe damage in the substantia nigra (SN), and also the presence of Lewy bodies in the surviving nigral cells [17]. However, it was shortly after the discovery of DA in the brain, that patients dying with Parkinson's disease were found to have severe loss of DA in the striatum, a target structure for release of DA produced in the nigral neurons [18, 19].

The synthesis of DA is regulated by the tyrosine hydroxylase (TH) enzyme (Fig 1A). TH catalyses the first and rate-limiting step of the catecholamine biosynthesis by converting tyrosine to L-DOPA, which is then decarboxylated to DA by the aromatic L-amino acid decarboxylase (AADC or DOPA decarboxylase, DDC). Once synthesized, DA is taken up into vesicles by the vesicular monoamine transporter (VMAT). DA mediates its effects by interacting with receptors located on post-synaptic neurons (D_1 and D_2 receptor families), but also binds autoreceptors modulating dopamine cell function. While part of the released DA is taken up by the DA transporter (DAT) to the cytoplasm, free-DA is metabolized by both monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) into homovanillic acid (HVA) and 3,4-dihydroxyphenylaceticacid (DOPAC) (see Fig 1A for illustration of DA synthesis).

DA-containing systems have been extensively investigated and divided into two major categories based on their anatomical position and connectivity. *The mesencephalic DA system* consists of the dopaminergic cells located in the SN (A9 group), DA neurons of the ventral tegmental area (VTA; A10 group), and the retrorubral nucleus (A8 group). Nigral neurons are mainly confined to the pars compacta and pars lateralis of the SN and give rise to the nigrostriatal and dorsal mesostriatal pathways, whereas the VTA cells and the retrorubral

nucleus form the ventral mesostriatal, mesolimbic and mesolimbocortical pathways. *The diencephalic DA system* includes four topographical subgroups called caudal diencephalic (A11), tuberal (A12), dorsal hypothalamic (A13), and the rostral periventricular (A14) cell groups that project to spinal cord, hypothalamic, or pituitary target areas (see ref. [20] for a detailed anatomical description of the DA systems in the central nervous system).

Post-mortem analysis of brains from PD patients revealed the fact that specific subpopulations of dopaminergic neurons are more vulnerable [21, 22], and that the parkinsonian neurodegeneration is not simply an accelerated form of cell loss that could be seen due to normal aging [23, 24]. Dopaminergic neurons located in the ventral mesencephalon, particularly the cells located in the substantia nigra pars compacta (SNc), are predominantly affected, and their degeneration underlies the dramatic decrease in dopaminergic innervation in the striatum. The first symptoms usually appear when about 50-60% of the neurons in the SNc and about 60-80% of the striatal DA contents is lost [24-26]. While the degeneration at SNc level is particularly pronounced, other dopamine-containing cell groups such as the VTA and the peri- and retrorubral areas are affected to a lesser extent, and the central grey substance remains nearly intact [27]. The cell loss in the SNc is heterogeneous since it predominantly occurs in the ventrolateral part explaining the specific pattern of denervation at the striatal level. The neurons that project to the putamen, the dorso-lateral and caudal part of the striatum involved in motor functions, are more affected than those projecting to the caudate nucleus (ventro-medial and rostral striatum) involved in cognitive tasks. Whereas the putaminal DA depletion reach to about 95% in the advanced disease, the corresponding decrease measured in the caudate nucleus is around 60-70% [25]. Neurodegenerative changes can also be found at the level of the locus coeruleus, nucleus basalis of Meynert, pedonculopontine nucleus, cerebral cortex and spinal cord, leading to neurochemical alterations in the serotoninergic, noradrenergic and cholinergic neurotransmission systems. These multiple neurotransmission deficiencies, however, seem to occur in the late stages of the disease, whereas other events such as changes in the glutamatergic activity at several level of basal ganglia can also be observed earlier in the disease.

Treatment strategies

Pharmacological therapy - Considerable efforts have focused on developing treatment approaches that provide antiparkinsonian benefits. Pharmacological dopamine replacement strategy, by using the DA precursor L-DOPA, has been the major treatment since its introduction in 1967 [28]. However, even if the symptomatic benefits of this medication are remarkable in the early stages of the disease, the majority of PD patients develop motor complications during long-term therapy. Indeed, with disease progression, these complications consist of marked swings between immobility and mobility (on-off motor fluctuations), involuntary movements (dyskinesia) but can also produce neuropsychiatric complications and non-motor fluctuations [7]. Main factors responsible for such side effects have been speculated to be directly related with the increasing dopaminergic nerve terminal loss and a pulsatile stimulation of DA receptors due to the short plasma half-life of L-DOPA [29-31]. Because of their longer plasma half-life, dopaminergic agonists have been proposed as a strategy to induce symptomatic benefits without adding severe motor complications. Compounds actually existing and used either as mono- or multitherapy (i.e. associated with low doses of L-DOPA), already contribute efficiently to counteract motor symptomatology of PD patients [31-33].

Because of evidence arguing that non-dopaminergic alterations are associated with the characteristic nigrostriatal neurodegeneration in PD patients, other options have been evaluated as alternative strategies in the treatment. Pharmacological approaches, targeting glutamate receptors, particularly the N-methyl-D-aspartate (NMDA) receptors, have been investigated and showed beneficial effects in both reversing the motor symptoms, and also the L-DOPA-induced dyskinesia in patients with motor complications [34, 35]. Use of these agents has been however limited because of adverse effects associated with non selective blockade of NMDA receptor function. Development of more potent and selective pharmaceuticals for both ionotropic and metabotropic glutamate receptors holds promise for future therapeutic approach.

Cell therapy – Fetal ventral mesencephalic tissue transplantation based on the rationale of restoring dopaminergic neurotransmission in the striatum has been investigated as a novel strategy for Parkinson's disease [36]. Clinical data, based on observations in about 350 grafted-patients, demonstrated that embryonic human nigral neurons were able to survive, integrate and function over a long time in the brains of parkinsonian patients [37]. However, although grafts can induce symptomatic relief, the clinical outcome is variable. Current

efforts are focused on optimization of cell therapy for best possible outcome and to achieve it consistently. In particular increasing graft efficiency and decreasing functional variability, selection of patients that are most suitable to receive striatal dopamine cell transplants, placement of the fetal mesencephalic tissue to reach the best striatal reinnervation and best clinical outcome with no side effects, and standardization of the procedures for preparation of the graft tissue are currently debated issues [37, 38].

Available clinical and experimental data indeed point to the fact that transplant-induced recovery is significantly better in younger than older subjects, suggesting that age and disease severity must be considered as important factors in the functional efficacy of the graft [39-42]. Moreover, since outgrowing axons seem to extend 2-3 mm from the transplantation site graft placement also appear as a critical parameter. DA depletion is known to be widespread, involving various structures, from the putamen to other parts of basal ganglia, limbic forebrain and cerebral cortex. Since this neuronal loss differs from one patient to another, placement of the dopaminergic transplants has to be personalized (for review, see ref. [37]). Composition and preparation of the graft tissue also appears as a critical factor for optimization of graft-induced functional recovery. Indeed, procedures for dissection of the fetal brain material and preparation of the tissue for transplantation have an impact on the outcome of the grafting. For example, injection of cell suspension is more favorable than solid tissue grafts since suspension grafts appear to induce less immunogenic responses, possibly due to the fact that the blood capillaries within the graft belong to the host rather than the donor tissue, as would be the case in solid grafts. Finally, appropriate long-term immunosuppressive treatment appears to be necessary to ensure the full functional potential of the transplants.

Huntington's disease

Huntington's disease (HD) is an inherited, autosomal dominant, neurodegenerative disorder characterized by involuntary choreiform movements, cognitive decline, and a progressive neuronal degeneration primarily affecting the striatum. At present there is no effective therapy, even palliative, against this disorder. The gene responsible for the disease has been localized on the short arm of chromosome 4 [43] and the molecular defect recently identified [44] as an abnormal repeat of CAG triplets in the 5' coding region of a gene (*IT15*) encoding a protein (huntingtin) with unknown function. Despite the intense search for a cell pathology attached to this molecular defect, the mechanisms leading to neurodegeneration in HD still remain largely speculative. Nevertheless, recent studies have suggested that abnormal interactions between the mutated huntingtin and other proteins could be involved in the pathogenesis of HD. Thus, huntingtin has been shown to interact with several proteins including a cytoplasmic protein that associates with microtubules, mitochondria, and synaptic vesicles (HAP-1; [45]), glyceraldehyde phosphate dehydrogenase (GAPDH; [46]), an unidentified calmodulin associated protein [47], an ubiquitin-associated protein (HIP-2; [48]), and a protein homologous to the yeast cytoskeleton-associated protein sla2p (HIP-1; [49]). These observations suggest that alterations in glycolysis, vesicle trafficking, or apoptosis could all be pathological mechanisms involved in HD. However, direct and indirect evidence for defects in mitochondrial energy metabolism (complex II–III deficiency) has been increasingly compelling over the past decade [50, 51]. It may be then conceivable that a complex interplay and possibly a direct link exist between HD mutation, mitochondrial impairment, excitotoxicity/apoptosis, and striatal neurodegeneration.

Clinical features

It is beyond the scope of this chapter to give a full description of HD and the reader is referred to review articles on the topic for more detailed information [52, 53]. However, a brief summary of the most relevant neuropathological and clinical features of HD will be given below. HD is characterized by abnormal choreiform movements, cognitive deficits, and psychiatric manifestations associated with progressive striatal atrophy. The onset, progression, and clinical expression of HD are variable even though it occurs in general during adulthood. In the common form of the disease, clinical symptoms develop very rapidly after onset and compose a three-part picture with motor symptoms, initially

characterized by hyperkinesia evolving to bradykinesia; psychiatric disturbances, with aggressiveness and depression; and profound cognitive impairment. With the exception of patients bearing the largest triplet repeats (juvenile variant) who normally become clinically overt within a few years after birth, most HD patients start to express motor abnormalities at ages 30–40.

In the most common form of the disease, motor disabilities progress over a 10–15-yr period from a hyperkinetic to an akineto-rigid syndrome. Typically, the earliest motor signs are eye movement abnormalities, followed by the progressive appearance of orofacial dyskinesias; dyskinesias involving the head, neck, trunk, and arms; and finally chorea [54]. As the disease progresses, choreiform movements may disappear, the initial hyperkinetic syndrome being progressively replaced by a more hypokinetic syndrome in which bradykinesia, rigidity, and dystonia may predominate [55]. In the juvenile variant, choreiform movements are generally absent, whereas dystonia and bradykinesia can been seen in the early stages of the disease, evolving rapidly to rigidity and severe hypokinesia.

Comparative neuropsychological testing of patients with HD, Parkinson's disease with dementia, or Alzheimer's disease points to HD as a model of subcortical dementia, even if this concept has been a matter of controversy (see ref. [56] for discussion). Nevertheless, the cognitive deficits observed in HD are very similar to those observed following lesions of the frontal cortex and perseverative behavior as well as severe impairment in set-shifting strategies ("cognitive flexibility") are key features of the frontal-type HD syndrome.

Neuropathological findings

The most striking neuropathological manifestation of HD is the progressive degeneration of the striatum (caudate-putamen complex). The degree of striatal atrophy has been used by Vonsattel and collaborators [57] to categorize HD brains into five different grades (from grade 0, no striatal pathology, to grade 4, severe caudate–putamen and nucleus accumbens atrophy). At this latest stage of neurodegeneration, atrophy is also readily observed in the cerebral cortex (frontal and prefrontal areas), pallidum, subthalamic nucleus, various thalamic nuclei, and substantia nigra. Interestingly, all these areas have in common that they belong to the basal ganglia circuitry and, as such, are directly or indirectly connected to the striatum. However, only the severity striatal atrophy has been shown to correlate with the severity of psychiatric and motor symptoms [58].

An interesting feature of HD striatal pathology is that not all striatal cells are equally affected by the degenerative process [59]. There is a preferential degeneration of γ-aminobutyricacid synthesizing (GABA-ergic) medium-sized spiny neurons and a relative sparing of the other subpopulations of striatal cells, at least in the early course of the disease [60]. Consequently, concentrations of substance P and Met-enkephalin that co-localize with different subsets of GABA neurons in the striatum, as well as the post-synaptic D_1 and D_2 dopaminergic receptors are decreased [61-63]. Decreased numbers of neurons immunoreactive for calbindin D28k, a Ca2+-binding protein present in a subset of striatal medium-sized spiny neurons has also been reported. Interestingly, Golgi staining studies showed that before death many striatal GABAergic neurons show morphological abnormalities in moderate grades of HD [64]. Changes in immunoreactivity for calbindin or Golgi staining indicate the presence of proliferative dendritic changes early in the disease. These morphological changes include increased size and density of dendritic spines, recurving of distal dendritic segments, and short-segment branching along dendrites. Even within the subpopulation of GABAergic spiny neurons, all cells are not similarly affected by HD. A double gradient of striatal degeneration has been described in the HD striatum, one progressing in a dorsoventral direction and another in a caudo-rostral direction [57, 65]. As a consequence, the most vulnerable GABAergic neurons appear located mostly within the dorsal parts of both caudate and putamen nuclei. Another intriguing neuropathological characteristic of HD is the relative sparing of the medium-sized interneurons positive for NADPH-diaphorase and somatostatin [60]. The preservation of this subset of striatal interneurons is associated with an increase in somatostatin and neuropeptide Y concentrations [66]. The enzyme responsible for the labeling of the medium-sized aspiny neurons within the striatum using NADPH diaphorase activity turned to nitric oxide synthase (NOS) [67, 68]. This striking sparing of NOS-positive interneurons and the possible neurotoxicity of NO led to the hypothesis that these interneurons may have a causal role in the pathogenesis of HD [69, 70]. The large cholinergic interneurons are also spared in HD striatum, even though choline acetyltransferase activity and muscarinic receptors are significantly decreased, probably as a result of synapse loss due to the loss of neighboring neurons [71]. Concentrations of dopamine and its metabolites are not markedly decreased and tyrosine hydroxylase immunoreactivity is maintained in the HD striatum [72, 73]. Finally, it can be noted that all major striatal afferences (in particular the dopaminergic, glutamatergic and serotoninergic afferents) are relatively unaffected by the degenerative process [67, 74].

Another characteristic of HD neuropathology is the presence of intranuclear inclusion bodies and extracellular fibrillar deposition that consists of the ubiquitinated N-terminal part of huntingtin, the mutated protein in HD [75].

Hypothesis on cell death mechanisms

The exact link between the proteins potentially interacting with huntingtin and the pathological process occurring in HD remains to be clarified. However, one hypothesis is a potential toxicity of the N-terminal part of the mutated human huntingtin. It remains that the mechanisms underlying cell death in HD remain largely unknown. Though, a number of triggering events leading to neuronal death have been identified to date, such as excitotoxicity (a term designating death resulting from excessive activation of glutamate receptors). The possibility that excitotoxicity may play a role in the aetiology of HD was supported mainly by the finding that focal injection of glutamate receptor agonists into the striatum produced lesions with histological and neurochemical characteristics resembling those seen in HD [75]. This hypothesis was later refined by suggesting that early energy impairment observed in HD patients may lead to the overactivation of NMDA receptors and relentless excitotoxic neuronal death [76]. Experiments on neuronal cell cultures and laboratory animals in vivo have suggested that mild energy failure could indirectly produce activation of an NMDA receptor, triggering an excitotoxic cascade and subsequently, actual neurodegeneration. Defects in energy metabolism in HD patients have been demonstrated *in vivo* using positron tomography and nuclear magnetic resonance spectroscopy. Biochemical analyses of postmortem tissue samples from HD patients have consistently shown a decrease in activity of complex II–III (succinate dehydrogenase and ubiquinone cytochrome *c* oxidoreductase). The possibility that the defect in complex II–III activity seen in HD may have a causal role in the aetiology of the disease is also suggested by the fact that well-characterized cases with biochemical defects in succinate dehydrogenase are associated with preferential striatal degeneration [77, 78]. In addition, poisoning with the succinate dehydrogenase irreversible inhibitor 3-nitropropionic acid in humans results in striatal lesions [79].

Finding new therapies for HD

Although neuroleptics are used worldwide to reduce the frequency and intensity of the motor symptoms, today there are no specific drug-based treatments available for HD patients. Despite the lack of an exact understanding of cell death mechanisms in HD, major efforts are currently undertaken to find a cure. Two basic features of the disease allow two lines of therapeutic strategies to be designed: first the predominant degeneration in the striatum and second, the neuronal loss progression over years after onset of clinical signs. It may thus be possible to consider substituting missing striatal neurons in patients by homologous neurons that can replace them functionally and anatomically. This line of clinical research therapeutics is currently based upon the use of intracerebral grafting of fetal neural tissue. The safety of the procedure has now been demonstrated [80, 81]. In addition, some therapeutic efficacy has been shown with long-term stability of performances, and clinical improvements on some symptoms being observed following fetal neural allografting [81]. A multi-center study that will allow the involvement of a number of patients large enough to carry out a valid group analysis, and therefore the comparison of a treated group with a matched control one, is currently actively pursued.

In parallel, one can also consider treatments that would strengthen the natural defense of neurons and, therefore, allow threatened neurons to survive, at least for a longer time period. This is the case of studies based upon the use of neuroprotective drugs or trophic factors. Simultaneously, ex vivo gene transfer techniques using encapsulated genetically engineered cells have been used to test the neuroprotective properties of ciliary neuroptrophic factor (CNTF) in HD patients. In this context, intraventricular implantation of CNTF-producing capsules in HD patients for one year proved to be a safe procedure, with no noticeable adverse events [82]. This encouraging phase I trial will be followed soon by a phase II protocol. Other gene-based strategies include inactivation of the mutated gene expression using viral vector mediated delivery of silencing RNA directed against the mutated HD gene. This therapeutic strategy which is currently tested in animal models is facing a technical problem on how to disperse a sufficient amount of the viral particles over large brain regions such as cortical and/or striatal territories.

General concepts in Positron Emission Tomography

Positron Emission Tomography (PET) is a complex and costly technology. In principle PET imaging is based on the use of radiolabelled ligands (molecules that can either be receptor ligands, enzyme substrates, false neurotransmitters, etc), which can label specific molecular targets in living organisms following an intravenous injection. This approach is multidisciplinary in essence as it requires radiochemists to synthesize the positron-emitting molecules, pharmacologists and biologists to validate them as specific radiotracers *in vitro* and *in vivo* as well as various other expertises to be able to qualify these radioligands as radiopharmaceuticals, adapted for a clinical use. Nevertheless, PET imaging is unique in that it offers the opportunity not only to visualize but also quantify activity of cells in specific organs or tissues under examination, providing direct information on the chemistry and function of that area.

The principles of PET

Upon decaying, certain radionucleids emit positrons that travel only about a millimetre in tissue before being captured by an electron. Both particles spontaneously annihilate in a matter-anti-matter reaction, which produces two 511 KeV gamma-ray photons travelling in exactly the opposite directions (180° apart from each other). A ring of hundreds of scintillation crystal detectors surrounding the object to be imaged is wired to detect such coincident pairs of gamma rays, which in turn, allows the location of the annihilation event to be traced along the line connecting the two detector points. A computer uses this information to generate three-dimensional, cross-sectional (tomographic) images that represent the biological activity where the radiolabeled compound has accumulated. Modern clinical PET scanners are equipped with 25-32 detector rings, which can simultaneously acquire multiple adjacent images (up to 63), providing a 3-dimensional view of the body or whole organs such as the brain with an in-plane resolution of 4.5mm. After a complete scan, a mathematical algorithm applied by the computer corrects the collected data for scatter, attenuation, accidental coincidences, normalizes for the differences in detector efficiencies and reconstructs the spatial distribution of the radioactivity density inside the organ or the system under study. The resulting image can be displayed on a screen, stored and/or further analyzed and processed.

After injection of trace amount of a radiotracer, PET can map the distribution pattern of the radioactivity with a million-fold better sensitivity than other techniques. Its spatial resolution is adequate for most purposes and relates to the size and number of detector crystals used in the PET tomography. Typically, a PET volume element (voxel) has dimensions of one to few millimetres. PET has recently been miniaturized making it applicable to small animal imaging, but the technique is before anything a nuclear medicine imaging technique that is nowadays widely used in oncology as a highly sensitive mean to detect tiny tumours in the living body, including the brain.

Radiotracers for PET studies range from standard image displays that provide direct indices of physiological function to more complex kinetic analysis methods using compartmental modelling approaches which provide quantitative assessment of binding parameters (B_{max} and K_d) or V_{max} values. Depending on their biochemical properties, PET radiotracers may be divided into two categories. The first category includes non-specific radiotracers following a biochemical pathway and allowing for measurement of tissue extraction or metabolism. Radiotracers from the first category include ^{18}F-fluorodeoxyglucose (^{18}FDG) used as a tracer to map glucose metabolism in the brain and, using an appropriate compartmental modelling approach [83], calculate an absolute quantification of regional glucose metabolism *in vivo*. A second example is ^{15}O-labeled water ($H_2{}^{15}O$), which is used as a tool for the estimation of physiological parameters such as oxygen metabolic rate and cerebral blood flow. In addition, the short half-life of ^{15}O ($t_{1/2}$=2.1 min) allows for rapid sequential measurements (PET activation studies) of cerebral blood flow using $H_2{}^{15}O$. Radiopharmaceuticals from this category may be assessed using a single or two-compartmental plasma-tissue model. The second category includes specific radioligands involved in an interaction with a transporter or a receptor site. Radiotracers from the second category are used to study changes in density and affinity of central receptors, and are described in detail in the following section.

Applications of PET in neurodegenerative states

PET was the first scanning method to provide information on brain function. Several neurological and psychiatric diseases have been related to neurotransmitter and receptor disorders. Due to its high sensitivity, today PET is used to detect and quantify subtle abnormalities in neurotransmission or neurotransmitter levels in neurological disorders,

including among others stroke, epilepsy, Alzheimer's disease (AD), PD and HD. There is now a validated radiotracer adapted to the study of almost all major neurotransmitter systems (for a review see [84-86] such as the dopaminergic, noradrenergic, serotoninergic, cholinergic, GABAergic (GABA-A benzodiazepine receptor complex) as well as more recently glutamatergic [87] neurotransmitter system. The information provided by PET may vary from one pathology to the other because of variation in the primary degenerative defect involved in the pathology. For example, PET studies in PD have been focusing on molecular imaging of the dopaminergic system (either the DA metabolism, DA receptor occupancy, or the DA transporter density) whereas studies in AD have focused on general rate of metabolism in parts of the forebrain, inflammation and/or tracers for the amyloid plaques as surrogate markers of the pathology.

The role of PET imaging in neurodegenerative diseases has been multiple and complementary to that of other imaging modalities. One of the first application of PET is to help characterize the role of a given neurotransmitter system in important functions such as motor control and the presence of subtle dysfunctions in living patients (see ref. [88] for review). For example in PD, PET imaging has been used in at least four different approaches. The loss of dopamine terminal function as reflected by decreased levels of striatal and extrastriatal ^{18}F-DA (Fig 1b) or dopamine transporter ligand (^{11}C-PE2I; Fig 1c) has been correlated with motor disability as well as cognitive performance [88]. Second, alterations in locomotor and behaviour in response to focal intrastriatal dopamine replacement by implants of fetal mesencephalic cells or local trophic factor infusions has been correlated with restoration of dopaminergic function in treated patients [89, 90]. Third, alterations in the patterns of resting and activated blood flow and metabolism in PD patients following dopamine replacement therapies has been characterized by PET [91]. Finally, dopamine release in striatal and also extrastriatal (mostly cortical) areas during task performance or pharmacological challenge has been shown using the indirect ^{11}C-raclopride PET approach [92, 93], an *in vivo* PET technique based on the competition between the ^{11}C-radiotracer and the endogenous levels of dopamine either released by task or pharmacological challenges (amphetamine, methylphenidate, L-DOPA) or even under basal conditions [94] (Figs 1d, and 2a,b,c).

Another important application of PET in neurodegenerative disease has also been to help establish a better diagnostic of uncommon forms of neurodegenerative disorders. For example, PET coupled to the use of dopaminergic pre- and post-synaptic markers like ^{18}F-

DOPA and ^{11}C-raclopride has been used to differentiate between typical PD in which no major loss in post-synaptic D_2-receptors occurs and other parkinsonian syndromes such as striatonigral degeneration or Steele-Richardson syndrome in which a significant loss of post-synaptic dopamine receptors can be demonstrated in the striatum. However, even if PET imaging actually provides functional information to confirm clinical diagnostic, it is worth noting that the use of PET on its own is not sufficient to diagnose a disease, as many syndromes may also be associated with similar alterations of a given radiotracer binding properties. In addition, using radiotracers other than the classical ^{18}F-DOPA, PET can also identify alterations in other catecholaminergic neurotransmitter systems and demonstrate that depression and anxiety in the patients is correlated with specific loss of DA and noradrenergic innervation in the limbic system [95].

The third very important application of imaging in neurodegenerative diseases such as PD or HD has been its use in the in vivo monitoring of disease progression. In this respect, PET imaging is nowadays frequently associated in clinical research to help quantify the annual loss rate of specific markers such as DA synthesis in PD or D_2-receptor expression in HD patients. Similarly, in HD patients, the initial PET studies carried out by Kuhl and collaborators [96] showed that cerebral glucose metabolism was affected in the striatum of HD patients. Marked decreases in glucose consumption could be attributable at least in part in symptomatic HD patients to striatal atrophy. However, substantial reductions in ^{18}FDG incorporation in the striata of patients presenting early symptoms with no gross atrophy were seen, suggesting that severe metabolic impairment could precede bulk tissue loss. In 15 at-risk patients, 6 showed striatal glucose utilization that was more than 2 standard deviations lower than the normal mean value [96]. These initial observations were confirmed by a number of other studies where alterations in striatal glucose metabolism were seen in early or presymptomatic HD patients [97, 98]. However, a decrease in striatal glucose consumption in at-risk patients was not always observed [99]. Moreover, it is worth noting that for several reasons mainly associated with ethical considerations, such studies may be confounded by the fact that the patients may have to take medications that most of the time compete competitively with the radiotracer used (for a review, see [100]).

The last point and probably the most awaited is the use of PET for the *in vivo* assessment of therapeutic efficacy. A major recent interest in the role of PET has been identified following completion of several large clinical trials evaluating the effect of chronic pharmacological treatment on disease progression. For example, in the REAL-PET study,

patients treated with the DA agonist ropinirole had a significant lower reduction of striatal and nigral ^{18}F-DOPA uptake over two years compared to the patients of the L-dopa treated control group [101]. Similarly restoration or striatal DA storage and release has been achieved by transplantation of fetal ventral mesencephalic tissue into the diseased brain. Evidence for graft survival and striatal reinnervation has been assessed post-mortem in patients that received transplants of fetal dopamine cells in the striatum [41, 42, 102, 103]. Graft viability has also been shown using PET [36, 41, 42, 104-106]. Importantly, the recovery of dopamine function being was found to be dependant on the amount of fetal tissue implanted [107]. Recently, the graft's functionality has been investigated by Piccini and colleagues, where DA release from the fetal DA neurons was visualized using ^{11}C-labeled Raclopride [89]. This was followed by a second study where the recovery in motor performance in grafted patients was correlated with reactivation of the frontal cortical areas using $H_2{}^{15}O$ PET, suggesting that functional recover required integration of the graft into host circuitry [108].

Similarly, using ^{18}FDG as a marker of hexokinase activity, PET has been used to study the metabolic effects of intrastriatal grafting in HD patients implanted with embryonic striatal neuroblasts [109]. As mentioned earlier, PET signature of HD is largely characterized by a severe hypometabolism in the striatum and several, mostly cortical, extrastriatal regions. Whereas, the striatal hypometabolism can certainly result from the severe cell losses occurring in these structures, the cortical hypometabolism may also result from a functional impairment of striato-cortical pathways, resulting from the progressive disappearance of striatal neurons in the striato-pallido-cortical loops. The PET study in grafted patients shed new lights on these issues by providing three major observations. First, it showed a parallel improvement of clinical status and striatal metabolism in 3 grafted patients (over the 5 that were involved in this small clinical trial) demonstrating that fetal grafts in the striatum of HD patients are able to restore some striatal function, in otherwise metabolically impaired patients. Secondly, the same PET data showed that the extent of the cortical hypometabolism observed before grafting was either reduced or stabilized in the same 3 patients showing clinical improvement. Conversely, in the two patients who were not clinically improved, striatal and cortical hypometabolism worsened. These results support the fact that the clinical benefit provided by the grafts in the first three patients is related to the functional improvement in the impaired striato-cortical loops. Of major interest in the context of HD, the reversibility of the cortical hypometabolism after striatal grafting (at least at an early

stage of the disease) means that it is not related only to the degeneration of cortical neurons, providing support to the rationale for intrastriatal grafting in HD

The third finding in this study was that striatal grafts displayed heterogeneous anatomic and metabolic profiles both within and across patients which may provide a mean to better understand the relationship between metabolic profile and functional recovery after cell therapy. The explanation of this heterogeneity in graft development and metabolism remains speculative. It might be related to the number of surviving cells and to the proportion of striatal tissue included in each implant or to the degree of reconnection of striatal grafted tissue with the host systems. It remains that the FDG uptake is mainly related to synaptic activity and that clinical improvement was observed only in the patients whose grafts induced an increase of global striatal and cortical metabolism.

General concepts in Magnetic Resonance techniques

The nuclear magnetic resonance (NMR) technique is built on the principle that every magnetic nucleus (also those in biological organisms), placed in an outer magnetic field, might be excited by applying a short pulse of energy. The specific frequency for each nucleus (called the Larmor frequency) depends on the gyromagnetic ratio of the nucleus, the field strength and interactions with surrounding nuclei. The resulting signal can be visualized in two different ways (Fig. 3). When the frequency spectrum of the signal from the whole, or part of an object is presented, the technique is called *magnetic resonance spectroscopy (MRS)* (Fig. 3c,d), while mapping the intensity of the signal in space is referred to as *magnetic resonance imaging (MRI)* (Fig. 3a-g). The relaxation of a paramagnetic nucleus can be investigated looking at two components of the relaxation, the spin-lattice relaxation (T1) or spin-spin relaxation (T2). Using different time intervals between excitation pulse and detection (termed echo time; TE) and/or between repetitions (TR), the signal will have different contribution ratio between T1 and T2 relaxations. We therefore refer to the resulting image as "T1-weighted" when the signal is formed mainly by contribution from T1 relaxation (Fig. 3a) and conversely "T2-weighted" when T2 relaxation is the dominant factor. Note that in reality all images have some contribution from both sources, and one can never separate them totally.

Due to the fact that water constitutes 50-70% of all soft-tissue-mass in the body, the MR signal from the hydrogen atom (^{1}H) gives by far the highest signal of all nuclei. Based on the cell composition in different parts of the brain, the water protons have a different microenvironment and therefore behave slightly differently. For example, in voxels containing myelinated axons (i.e., white matter tracts), there is a higher concentration of lipids compared to voxels containing cell bodies and non-myelinated axons, as is the case in grey matter. Using specific TE and TR values we can construct an image of the brain with high contrast between the white and grey matter. The more heterogeneous the tissue composition is the better potential for high contrast. As a result, MRI has the strength of giving high-resolution (sub-mm scale) morphological images of the brain matter [110]. MRS, on the other hand, can give us information on the chemical composition of areas in the brain. Each peak in the MR spectra corresponds a specific resonance-frequency (chemical shift) and represents a distinct position of a paramagnetic nucleus, providing an NMR fingerprint of each molecule (Fig. 3c,d). Although ^{1}H is the most commonly used for MRI and MRS today, it is possible to acquire signal from various other magnetic nuclei, including, e.g., carbon

(^{13}C), fluorine (^{19}F), and phosphorus (^{31}P). The sensitivity and the natural abundance of these nuclei vary largely relative to ^{1}H. While natural abundance of ^{1}H in hydrogen atoms is 99.99%, only 1.1% of carbon atoms are made up of ^{13}C. Therefore, the ^{13}C signal from biological organisms is several orders of magnitude smaller than ^{1}H.

Major metabolites such as glutamate and lactate can be detected, identified and quantified using the MRS techniques. Some of the compounds quantifiable by ^{1}H MRS in the brain are N-acetylaspartate (NAA), lactate, glutamine/glutamate (Glx), creatine (Cr), phosphocreatine (PCr), choline and *myo*-inositol [111]. The concentration, and the uniqueness and the width of the MRS peaks produced by the compounds are the major factors that determine the smallest detectable amount of a substance in the brain. As a guideline around 0.5-1 mmol is the lowest amount detectable in the clinic during a regular ^{1}H-MRS examination of the brain. Detection of minor metabolites present at low concentrations in the brain is hampered by limited sensitivity, spectral overlap with signals from major metabolic components, and structural background from macromolecules.

Functional MRI (fMRI) is another NMR based imaging modality, which uses the blood oxygen level dependant (BOLD) parameters to infer rain activity. The BOLD principle is based on the fact that deoxyhemoglobin is paramagnetic (i.e is attracted to a magnetic field) whereas oxyhemoglobin is only weakly diamagnetic. An increase in the concentration of deoxyhemoglobin in a voxel will lead to inhomogeneities in the magnetic field and thereby result in a decrease in the T2* [112]. When an area of the brain is activated, there is an increase in oxygen consumption that results in a slight initial increase of deoxyhemoglobin, but this increase is very soon replaced by a larger decrease due to an oversupply of oxygenated blood. Thus, by using a series of echo-planar image (EPI) stacks acquired in interleaving on and off states of the stimulation of interest, regional elevation in signal intensity can be correlated to activation in defined regions of the brain, for the most part in the cortical areas [112].

Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) are comparably new techniques within the field of MR and are based on the Brownian motion of water molecules in the tissue. While the water molecules display homogenous (isotropic) diffusion properties in a pure liquid environment, in the heterogeneous brain tissue, movement is not free in all directions but follow a pattern based on the tissue structure (anisotropic diffusion). It is believed that the water molecules diffuse more easily along the fiber-bundles in the white matter than perpendicular to them [113, 114]. Using DWI acquisition techniques, the

mean travelling distance in each axis can be acquired in diffusion maps from which one can calculate the apparent diffusion coefficient (ADC) for each voxel. DTI, on the other hand, uses quantitative acquisition of the diffusion in six or more directions in order to calculate a tensor that describes the shape of a diffusion ellipsoid in that voxel. The more elongated, cigar shaped, the ellipsoid is the more anisotropic is the diffusion. This information can then be displayed in a 3D vector field that can be used as a basis for tractography (Fig. 3e) reconstruction of tract trajectories) and thereby visualize the extent of major fibre tracts in the brain [114]. Using this technique, distortions in major fiber tracts can be investigated (Fig. 3f).

Contrast agents and labelling compounds have turned out to be one of the most difficult aspects of MR-imaging, but a few different alternatives exist with Gadolinium (Gd) chelates being the most used contrast agents to date. Gd-contrast agents work by interaction between the Gd-chelate and the surrounding water molecules. This interaction accelerates the T1 relaxation of the water molecules and thereby increases the signal at certain pulse sequences. Gd-contrast agents are successfully used in applications such as visualization of brain tumors (Fig. 3g), localization of blood-brain barrier disruption (e.g. in stroke) and angiography (Fig. 3h). In fact, about one third of all MRI examinations performed in the clinic today make use of contrast agents with Gd-chelates being in great majority [115]. All clinically approved Gd-complexes are intravenously administered, non-specific agents without any cellular or molecular recognition capabilities. To further advance the possibilities of MRI in cell therapy and other MR applications, novel specific contrast agents must be developed.

MR in neurodegenerative states

As for other neurodegenerative diseases, PD is associated with subtle structural changes that are difficult to detect by traditional MRI techniques. Volumetric MRI indicates only modest regional morphological changes in extranigral brain-structures of patients with advanced PD, whereas reliable MRI markers of underlying neurodegenerative abnormalities are yet to be developed [116]. Based on the anatomical MRI images and optimized voxel-based morphometry it has been shown that changes occur on many areas of gray-matter e.g. in the hippocampus, anterior cingulate gyrus, and superior temporal gyrus [117, 118]. Volumetric MRI analysis could also prove to be important for differential diagnosis between typical idiopathic PD and the striatonigral variant of multiple system atrophy (MSA-P). This differentiation is otherwise

difficult due to similar signs and symptoms especially at disease onset. There appears to be a larger decrease in the putaminal volume in MSA-P than in that of PD patients, compared to control [119]. Similar to this, other groups found that differences in the trace of diffusion tensor values or magnetization transfer of the putamen and globus pallidus also successfully differentiate between MSA-P and PD [120, 121]. Similar studies have been conducted on HD patients to show global decrease of brain volume as well as localized decrease in volume coupled to increase in mean ADCs in the caudate nucleus compared to healthy control subjects [122]. Moreover, MRI examinations in patients with early onset HD showed abnormalities in signal intensity indicating necrosis and a rapid process of degeneration [123, 124].

Many studies have been conducted using MRS on PD and HD patients with the hope to find patterns of metabolites that could aid in early diagnosis and understanding of the disease. However, clinical ^{1}H MRS studies (at 1.5 T field strength) have failed to detect regional metabolite changes in multiple brain structures and tissues in patients with PD, other than some loss of creatine in the region of the substantia nigra, which could possibly be caused by pharmacological treatment [116]. The major disadvantage with this approach is that none of the more specific neurotransmitters and metabolites of interest can be detected due to the low available concentrations and the low sensitivity of the technique. Therefore all studies have looked at only major metabolites such as NAA, Cr and PCr, (Cr and PCr cannot be separated in ^{1}H-MRS), or lactate. In HD patients, evidence for alteration in energy metabolism comes from the study of cerebral concentrations in lactate using MRS, in which substantial increases were seen in the striata but also in the occipital cortex, a brain region with no ongoing process of neurodegeneration, [125, 126]. This observation points to an alteration of oxidative metabolism in HD, in line with the results of several biochemical studies demonstrating an alteration of the mitochondrial complex II activity in HD [50, 127]. NAA has also used as a marker for viable neurons but is very unspecific and has also been shown to exist in high amounts in e.g. astrocyte progenitor cells [111]. Creatine has been used as an indicator of defective energy metabolism but has an even less defined role in neurodegeneration [128]. Even though findings of changes in the level of these metabolites have been detected on diagnosed patients, there is very low potential for ^{1}H-MRS to become an important tool in the diagnosis of neurodegenerative diseases without major development of labelling compounds or ways to dramatically increase sensitivity.

Imaging in cell-therapy

The main modality of magnetic resonance that has been used for cell-based therapy in the brain is anatomical MRI (Fig. 4a,e). The correct placement of the graft is very crucial for a successful outcome of cell based therapy and here the MRI with is excellent soft tissue contrast has become a very valuable tool. Stereotactic guidance using MRI (Fig. 4d) is not without problems though. Due to the way the three-dimensional image is built up in the MRI using magnetic gradients, susceptibility artifacts or magnetic field wrapping can lead to distortions displacing the structure of interest [129, 130]. Under well-controlled circumstances with a careful shimming, studies have shown the MR image to be correct down to half a millimeter, so for most applications it is still very useful [131, 132]. In post-surgery imaging in combination with PET (Fig. 4c,g), MRI can help estimate the success of the placement by looking at local changes in T2 relaxation in relationship with graft function as estimated by PET. Edema caused by the surgical procedure, which might have a negative effect on the graft survival, can also be detected. The use of functional MRI has given very few new insights into the neurodegeneration in the basal ganglia or the survival and function of neural transplants. One study suggests that there is a small but detectable increase in blood oxygenation level of areas with cell transplants in response to specific motor tasks [133]. Studies have also been conducted using MRS on human fetal transplants but due to the low sensitivity of the technique and the non-specificity of the metabolites examined the findings from this study can only be interpreted as proof of graft survival [134].

Future Prospects of PET and MR Imaging

As mentioned earlier, the great versatility of PET as well as its exquisite sensitivity makes it the indisputable technique of the future for molecular imaging studies in experimental animals and human beings. Almost every of the neurotransmitter, enzymatic or receptor system can be (or will be) imaged in the future using PET. Besides the already large panel of ligands selective of the dopamine system, radiochemists have now developed and pharmacologists validated, hundreds of other radiotracers selective of various neurotransmitter systems such as the serotoninergic, noradrenergic, benzodiazepine, cholinergic, glutamatergic and glycinergic pathways [135]. In addition, selective inhibitors of various enzymes such as MAO-A, MAO-B inhibitors have been radiolabeled with positron-emitters. Even small inhibitory peptides or macromolecules like proteins, spiegelmers, aptamers or oligonucleotides can now be radiolabeled – the later paving the way to the *in vivo* quantification of gene expression [136].

One of the major challenges of the last years is to understand the molecular mechanisms underlying aberrant protein folding. Indeed, a key feature associated with the nigrostriatal degeneration is the presence of intracellular protein aggregates, known as Lewy bodies, which can be seen in the surviving DA neurons of the substantia nigra. Although, α-synuclein is recognized as the major component of Lewy inclusions, its exact role in the neurodegenerative process still remains not clearly elucidated [137, 138]. However, since the formation of aggregates has been shown to anticipate the occurrence of the symptoms, targeting characteristic components of these inclusions might be a clever way to assess the development of PD pathology. In the past few years, such *in vivo* detection approach has been particularly successful in studies conducted in AD patients using radiolabelled compounds suitable for amyloid imaging using PET [139-141]. As an example, clinical data using the ^{11}C-labelled Pittsburg Compound-B (^{11}C-PIB) nicely showed an increase in PIB retention in several brain areas such as the frontal, parietal, temporal and occipital cortices, but also in the striatum, providing information on amyloid deposits in AD patients [140]. Similar tracers might then also be particularly useful to detect neuronal abnormalities prior the onset of the symptoms in PD, as an early diagnosis, but also for the follow up of the evolution of disease. Ligands targeting specifically α-synuclein protein aggregates might hold promise in the development of such diagnostic approaches for PD, and complete the available markers already existing such as β-CIT, DTBZ or fluorodopa, as index of

presynaptic dopaminergic activity or ^{11}C-Raclopride as an index of postsynaptic D2 receptors reactivity [142, 143].

Beside these active developments of the PET technique on its own, the potential of PET may be further expanded by combining it with other *in vivo* techniques such as MR spectroscopy. Especially with the advent of high field MR spectrometers, which enable the direct quantification of up to 14 to 20 cerebral metabolites in the living patient brain, MRS gives access to various metabolites that are complementary to those accessible to PET such as various neurotransmitters (glutamate, aspartate), neuronal (N-acetylaspartate) and/or glial markers (alanine, glutamine). Perhaps more importantly, and unlike PET, MRS allows the non-invasive measurements of metabolic fluxes in the human brain based on the detection of label incorporation (such as ^{13}C) into large pools of metabolites. Taking advantage of the unique property of MRS that allows to specifically identify the molecule and the atomic position at which the isotopic label accumulates, pioneering studies have already demonstrated the feasibility of quantifying TCA cycling *in vivo* in neurons or astrocytes following infusion of ^{13}C-glucose or ^{13}C-acetate, as a substrate [144, 145]. Recent studies in a non-human primate model of HD show the interest of combining ^{18}F-FDG PET and ^{13}C-glucose MRS studies in the same animals to gain better insights into the metabolic consequences of mitochondrial impairment [146]. There is little doubt that in a near future, similar combination of PET/MRS studies will now be conducted in patients to enlighten the role of astroglia in supporting brain activity under diseased states. Similarly, MRS at field strength of 7T or higher may offer the possibility of identifying the degree of phenotypic maturation of fetal neuroblasts (characterized by their high lactate/NAA ratio) into, fully differentiated, adult neurons (characterized by their high NAA/lactate ratio).

It is important to remember that ^{13}C has a natural abundance of 1.1%. This could be seen as an advantage because one can conduct ^{13}C spectroscopy looking at the natural abundance of ^{13}C in a tissue, but at the same time it is a disadvantage when using labeled compounds enriched for ^{13}C because of the high background in the brain. In addition, a second major limitation of ^{13}C as a NMR nucleus is the low magnetic resonance sensitivity, which is only about 1.6% of that of ^{1}H. A paramagnetic nucleus that has very low natural abundance in the brain but has a higher sensitivity than ^{13}C would be very valuable. A good candidate for this is fluorine (^{19}F). There is no naturally occurring ^{19}F in the brain and 100% of all fluorine is paramagnetic. Moreover, the magnetic resonance sensitivity is of ^{19}F is 83% of that of ^{1}H making it a good candidate for labeling. Along this line, Higuchi and colleagues

have created an amyloidophilic congo red type compound containing ^{19}F [147]. This compound crossed the blood brain barrier, and showed low or no toxicity and good specificity for amyloid-ß plaques. Using ^{19}F-MRI, they were able to detect amyloid lesions, and visualized plaques occupying as little as 2.5% volume in some areas of the mouse brain. With the possible optimizations of this technique (using ligands with higher number of fluorine atoms and higher kinetics over the blood brain barrier) and the gain of the scaling factor to humans, this could prove to be a very cost effective method for pre-symptomatic diagnosis of AD. Similar approaches could be used for imaging of other processes in the brain e.g. in visualization of the dopamine system. Dingman and colleagues have recently published initial studies looking at the pharmacokinetics of a nine-^{19}F-tagged variant of L-DOPA [148, 149]. Though still in its infancy, MRI or MRS measurements using ^{19}F-labeled compounds could prove to be a very valuable addition to the PET ligands available today.

Figure Legends

Figure 1. Schematic drawing of a synaptic terminal of a nigrostriatal dopamine (DA) neuron and its post-synaptic target, striatal GABAergic projection neuron. Normal DA neurotransmission is displayed in panel A. Dietary tyrosine enters the brain via active amino acid transport. In the DA neuron, tyrosine is converted to DA in two steps: First and the rate-limiting step is the synthesis of L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (TH). The second step is catalyzed by aromatic aminoacid decarboxylase (AADC). Newly synthesized cytoplasmic DA is then captured in specialized vesicles by the vesicular monoamine transporter-2 (VMAT-2), and released into the synaptic cleft upon activation. On the post-synaptic side, DA binds to its specific receptors (D1 and D2 receptor families) to mediate its actions. DA is then either metabolized extraneuronally into homovanillic acid (HVA) by monoamine oxidase (MAO), or taken up by the pre-synaptic terminals by the DA transporter (DAT) to be re-vesiculazied or metabolized into 3,4-dihydoxyphenylaceticacid (DOPAC) by catechol-O-methyl-transferase (COMT). In panels B-D, three different PET ligands used for visualization of the DA system are illustrated. First, radioactive fluorine substituted L-DOPA (^{18}F-DOPA) can be administered systemically. Upon entrance into the DA neuron, the labeled precursor is converted to ^{18}F-DA and stored in vesicles, thus giving an estimation of total storage capacity (B). Another way to label the pre-synaptic neurons is to use a specific ligand (^{11}C-PE2I) for the DAT (C). Alternatively, we can label the post-synaptic DA receptors by using ^{11}C labeled Raclopride, a partial D2 antagonist. In the normal brain, DA and Raclopride will compete for the binding to the D2 receptors, and thus partially occupy receptor sites. ^{11}C-Raclopride can, therefore, be used as a measure of both pre- and post-synaptic neurons in this system.

Figure 2. ^{11}C-Raclopride PET is a useful tool to assess DA neurotransmission in healthy and parkinsonian brain. While, normally both DA and Raclopride partially occupy the D2 receptor sites located on the striatal neurons and give a specific signal proportional to ^{11}C-Raclopride bound to the receptors (A), in the parkinsonian brain (where DA neurons are lost) ^{11}C-Raclopride signal is increased (B). Conversely, binding to D2 receptors can be reduced by increasing the release of DA at the synapse (by for example administration of amphetamine), thus increasing the competition for the binding sites (C). Using the latter case scenario, in vivo DA release has been visualized in a grafted patient where DA release from the graft derived terminals were stimulated by amphetamine (see refs [89, 150] for further details).

Figure 3. Multi-modality non-traumatic imaging approaches suitable for the in vivo assessment of cell-based therapies. Panel A: T1-weighted magnetic resonance imaging displaying the brain anatomy of a patient suffering from Huntington's disease. Note the enlargement of the lateral cerebral ventricles and the bilateral atrophy of the caudate-putamen complex. Panel B: 18F-fluorodeoxyglucose positron emission tomography image of the very same patient's brain, displaying the regional cerebral metabolic rate of glucose. The PET image (coded in false colors with red displaying high metabolic rates and yellow to green and blue for respectively intermediate, low and very low metabolic rates) is superimposed to MR image taken at the very same brain level. In this transplanted patient, the striatal graft (intercept of the two white lines) is characterized by a high metabolic rate of glucose indicating viability of the transplanted neurons. Panel C and D: Nuclear magnetic spectroscopy spectrum acquired in vivo in the striatum of a primate. Depending on the strength of the magnetic field used, the technique makes it feasible to quantify up to 10 cerebral metabolites such as phosphocreatine (a high energy compound), glutamate (the excitatory neurotransmitter), glutamine (a glial marker), GABA (the inhibitory neurotransmitter), or N-acetyl-asparate (a marker of neuronal integrity). Panel E and F: Diffusion weighted imaging (E) and fiber tracking (F) in a human volunteer. In panel E, the fractional anisotropy in each voxel of the image is color-coded to display in 3-dimensions the main organization of the fiber bundles in the living human brain. In F, the main fiber bundles are extracted and represented in 3-dimensions. G. Multifocal glioblastoma multiforme T1-weighted coronal image after i.v. contrast injection. H. 3D inflow MR angiography (MIP) without contrast injection. Panels G and H are courtesy of Dr Elna-Marie Larsson at Lund University Hospital and have been acquired using the 3T Philips Intera MR scanner.

Figure 4. Schematic representation of the experimental paradigm used in brain imaging assessment of cell-based therapy in neurodegenerative diseases such as PD or HD. Pre-graft, patients receive of pre-graft MRI (A) and a baseline PET scan (B) to assess the patient's brain in term of anatomical and functional abnormality. These two data sets are merged together to generate a composite image (panel C) in which the function (in the present case glucose consumption rate) is superimposed on the anatomy. The pre-surgical MRI is also used to guide the stereotactical approach necessary to implant the cells into the targeted brain region (Panel D). Post-surgical MRI and PET scans (Panel E and F) are used to assess both the anatomical location of the grafted cells and their functional status. Again, merged

MRI/PET images (Panel G) are generated to better define the location of the grafted neurons into the host brain in relation to their biochemical status.

Frequently asked questions on brain cell therapy and imaging

1) Are PET and MRI sensitive enough to visualize grafted cells function or anatomy in vivo?

PET, which is based on the use of radiolabelled - positron emitting - compounds is known as the most sensitive method to detect externally trace amounts (around picomoles) of biotracer molecules. Published data clearly indicate that such sensitivity allows function of the grafted cells to be visualized and quantified, in an absolute manner, in the living brain of a variety of experimental animals (rats, primates, pigs) as well as in patients. This is particularly true for radiotracers like false neurotransmitters or enzyme substrates that can accumulate with time in the grafted neurons. However, when the tracers used are targeting membranous proteins such as neuroreceptors or transporters, present in limited number on the grafted cells, PET may not be sensitive enough to detect the transplanted cells, especially if these cells are widely distributed within the host brain. With the development of high resolution MRI methods, functional as well as anatomical imaging of individual grafted cells may become feasible – but this is still a largely unexplored field of research.

2) Will PET imaging still play a role when brain cell therapy is a routine clinical procedure?

Noninvasive brain imaging has been largely used in the past 10 years as a mean to monitor in vivo the loss of function occurring during the course of a neurodegenerative disorder and the progressive recovery following experimental therapeutics such as neuroprotective treatments or cell-based therapies. It is thus very likely that methods for monitoring of transplanted cells in vivo will continue to play a critical role in the future. Now that proof of principle for *in vivo* cell imaging in experimental and clinical trials has been obtained, efforts need to be made to develop new biomarkers with higher selectivity and sensitivity for the transplanted cells as compared to the host brain, to identify them unequivocally and follow their fate in the host brain.

3) Could brain cell therapy be the cure for neurodegenerative diseases in the near future?

Several pre-clinical and clinical studies have shown that brain grafting may be therapeutically efficacious in a number of disease conditions such as Parkinson’s disease, Huntington’s disease and ischemia. Multi-centre phase I/II clinical trials are now under way that should

establish the extent of therapeutic efficacy of cell-based therapies. Available data already demonstrate that clinical recovery following intracerebral grafting may be variable within and across patients. However, the elucidation of mechanisms which may be responsible for this within and inter-individual variability may help define the optimal conditions for brain reconstruction.

4) What are the mechanisms by which cell-based therapies may improve brain function?

So far, two different strategies for brain repair following cell-based therapies have been tested, depending on the brain pathology considered. In the case of Parkinson's disease for which restoring normal dopamine levels within the diseased caudate-putamen complex (striatum) may be sufficient to induce a significant (though still not optimal) clinical recovery, ectopic striatal implantation of dopamine-producing cells has been shown effective. This is likely permitted by the fact that dopamine acts in the striatum as a neuromodulator of motor control neural circuits, rather than acting as a neurotransmitter within specific point-to-point neuronal circuits. In the case of Huntington's disease, in which the neurodegeneration targets striatal GABAergic neurons that constitutively form part of the striato-pallido-thalamo-cortical circuits, the aim of neural grafting is to "recreate" the damaged circuit by replacing lost neurons in the circuit by new neurons. In both cases, intracerebral grafting has been shown equally effective – although the procedures still need to be improved to attain sufficient reproducibility to become a broadly clinically useful therapy

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Figure 1

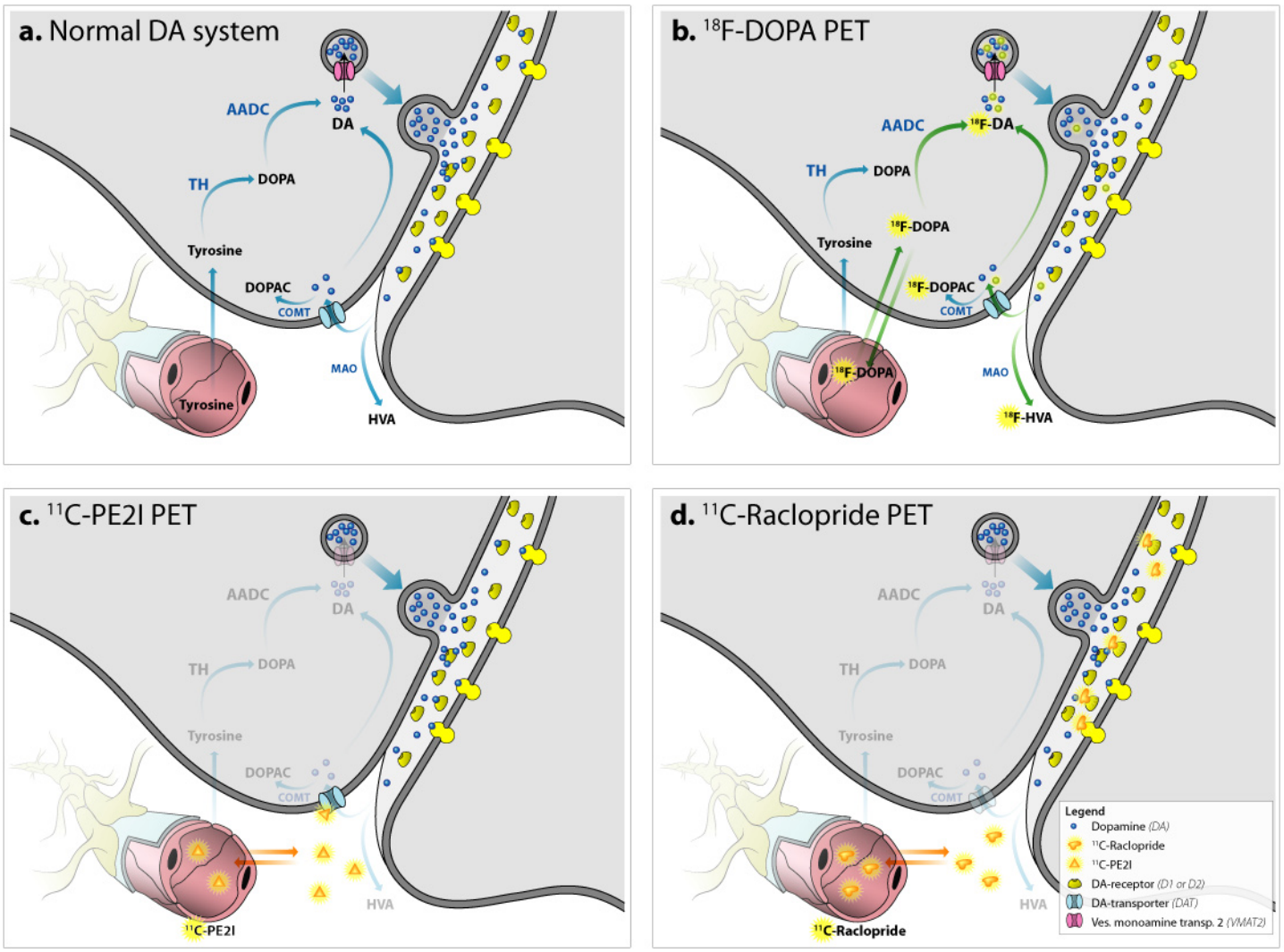

a. Normal DA system
AADC
DA
TH
DOPA
Tyrosine
DOPAC
COMT
MAO
HVA
Tyrosine
b. 18F-DOPA PET
AADC
18F-DA
TH
DOPA
18F-DOPA
Tyrosine
18F-DOPAC
COMT
18F-DOPA
MAO
18F-HVA
c. 11C-PE2I PET
AADC
DA
TH
DOPA
Tyrosine
DOPAC
COMT
HVA
11C-PE2I
d. 11C-Raclopride PET
AADC
DA
TH
DOPA
Tyrosine
DOPAC
COMT
HVA
11C-Raclopride
Legend
Dopamine (DA)
11C-Raclopride
11C-PE2I
DA-receptor (D1 or D2)
DA-transporter (DAT)
Ves. monoamine transp. 2 (VMAT2)

Figure 2

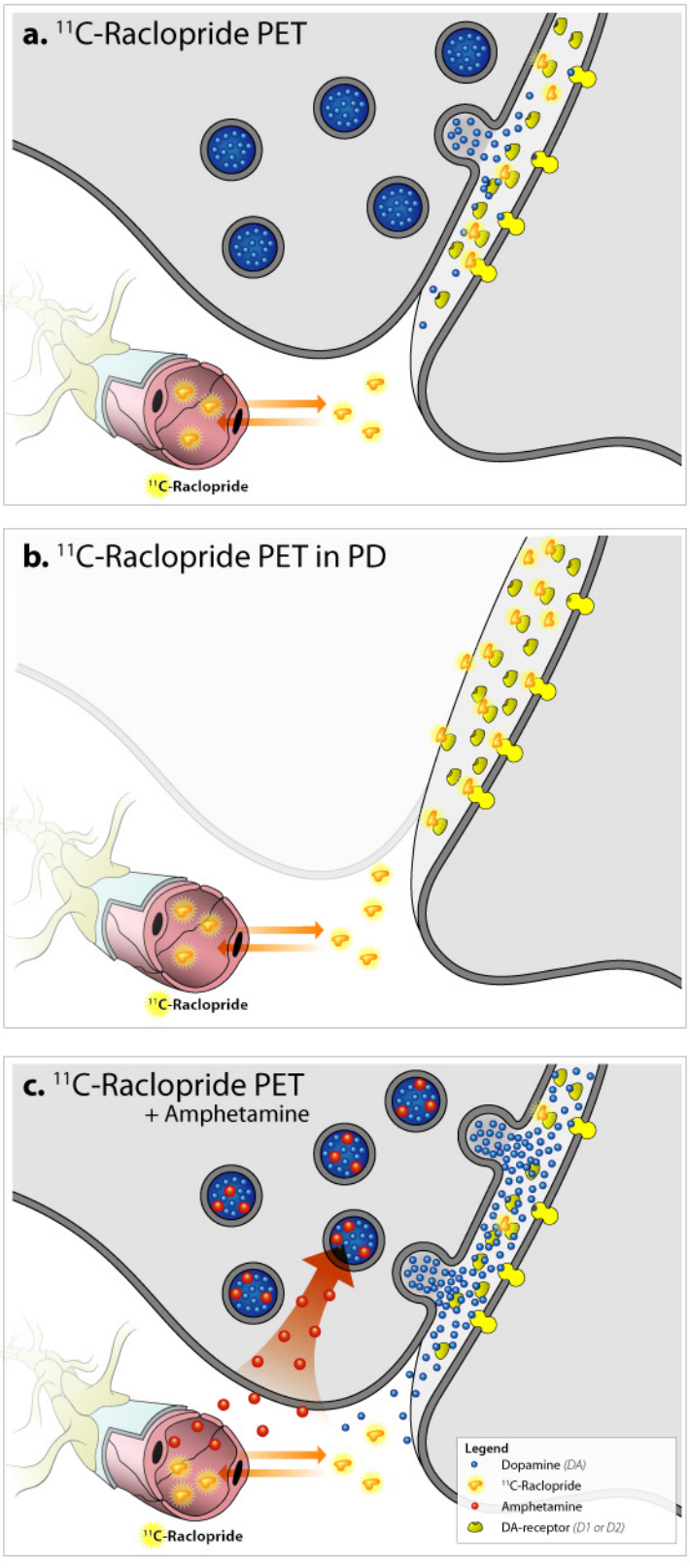

a. 11C-Raclopride PET
11C-Raclopride
b. 11C-Raclopride PET in PD
11C-Raclopride
c. 11C-Raclopride PET
+ Amphetamine
11C-Raclopride
Legend
Dopamine (DA)
11C-Raclopride
Amphetamine
DA-receptor (D1 or D2)

Figure 3

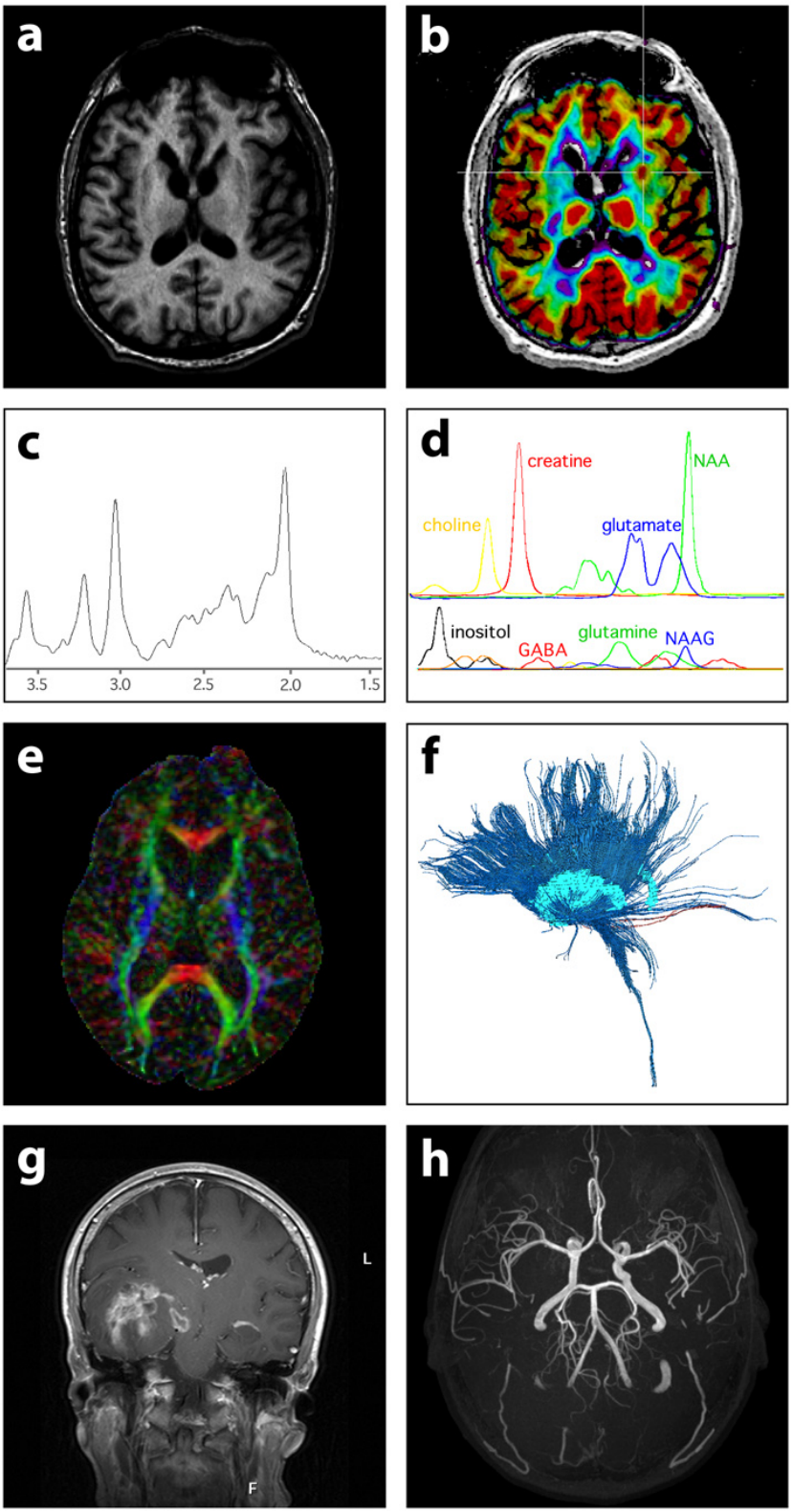
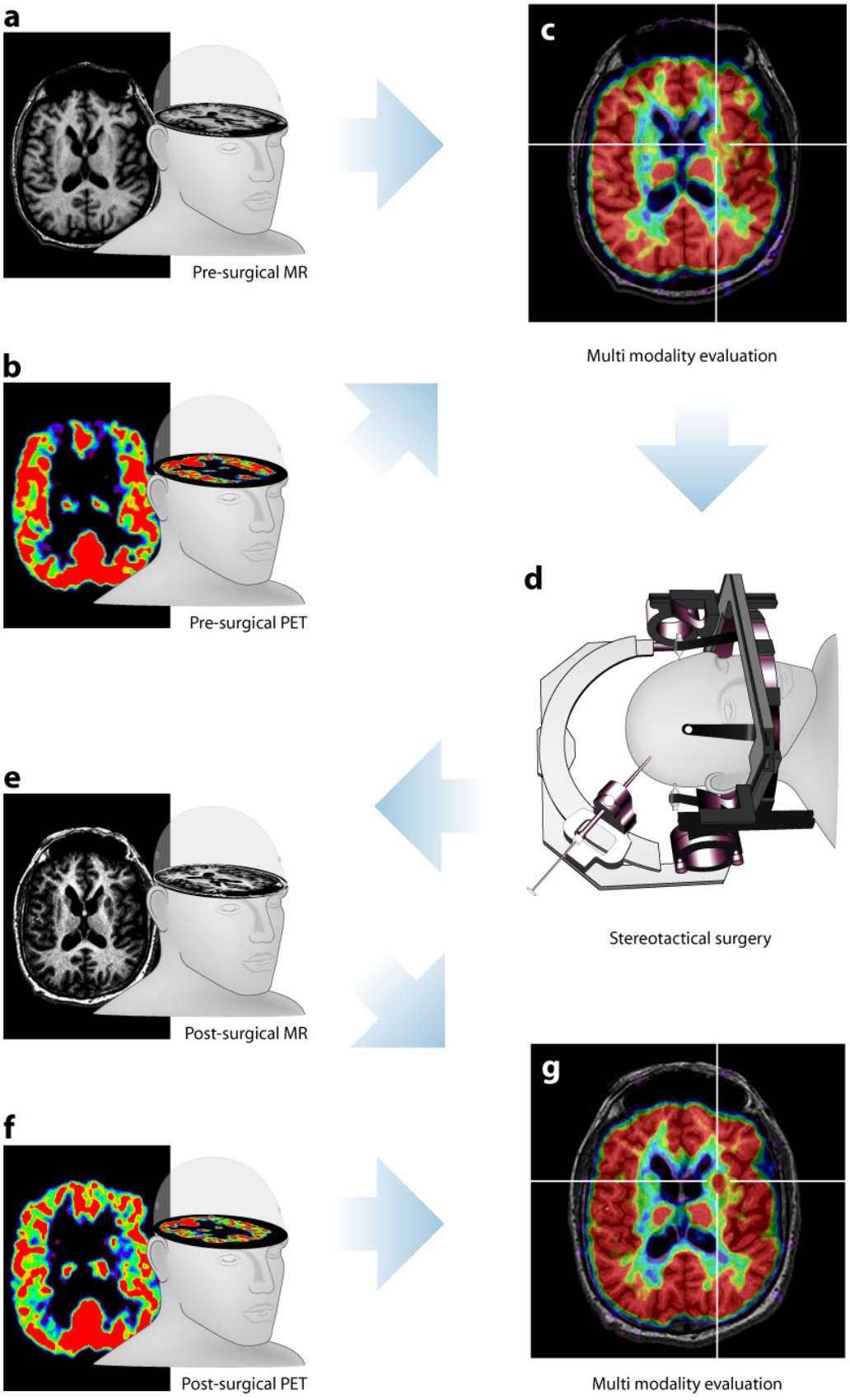

a
b
c
3.5
3.0
2.5
2.0
1.5
d
creatine
NAA
choline
glutamate
inositol
GABA
glutamine
NAAG
e
f
g
L
F
h

Figure 4


a
Pre-surgical MR
c
Multi modality evaluation
b
Pre-surgical PET
d
Stereotactical surgery
e
Post-surgical MR
f
Post-surgical PET
g
Multi modality evaluation