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Imaging and spectroscopic approaches to probe brain energy metabolism dysregulation in neurodegenerative diseases

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3 **Imaging and spectroscopic approaches to probe brain energy metabolism dysregulation in**
4 **neurodegenerative diseases**
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49 **Running title:**
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51 Imaging of energy metabolism in brain diseases
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Abstract

Changes in energy metabolism are generally considered to play an important role in neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), and Huntington's diseases (HD). Whether these changes are causal or simply a part of self-defense mechanisms is a matter of debate. Furthermore, energy defects have often been discussed solely in the context of their probable neuronal origin without considering the cellular heterogeneity of the brain. Recent data point towards the existence of a tri-cellular compartmentation of brain energy metabolism between neurons, astrocytes, and oligodendrocytes, each cell type having a distinctive metabolic profile. Still, the number of methods to follow energy metabolism in patients is extremely limited and existing clinical techniques are blind to most cellular processes. There is a need to better understand how brain energy metabolism is regulated in health and disease through experiments conducted at different scales in animal models to implement new methods in the clinical setting. The purpose of this review is to offer a brief overview of the broad spectrum of methodological approaches that have emerged in recent years to probe energy metabolism in more detail. We conclude that multi-modal neuroimaging is needed to follow non-cell autonomous energy metabolism dysregulation in neurodegenerative diseases.

Keywords

Brain Imaging – MR spectroscopy – Neurodegeneration – Astrocytes – White matter/oligodendrocytes

Impaired energy metabolism is a hallmark of neurodegenerative diseases

Most pioneering studies in neurodegenerative diseases (ND) and energy metabolism have focused on alterations of glucose catabolism through glycolysis and the production of reducing equivalents in the tricarboxylic acid (TCA) cycle that feeds the respiratory chain, eventually generating ATP using the protonic force in mitochondria. The working hypothesis was that ND are associated with the disruption of ATP production and that the loss of ATP bioavailability may be instrumental in precipitating dysfunction and neurodegeneration in ND. The present manuscript does not intend to provide an extensive review of the pioneering works that investigated this hypothesis; however, key findings can be reminded that help to better understand the evolution of concepts and views on the possible role of deficits in energy metabolism in ND, focusing on three main neurodegenerative diseases (ND), namely Alzheimer's (AD), Parkinson's (PD), and Huntington's diseases (HD).

There was renewed interest in this hypothesis when experiments performed with primary cultures of neurons showed that neuronal death, called "excitotoxicity" (caused by excessive glutamate release, overstimulation of ionotropic glutamate receptors and Ca^{2+} overload), was markedly stimulated by subtoxic blockade of energy production ¹. Similar findings in animal models led to the hypothesis of "indirect excitotoxicity", where partial energy defects can produce progressive cell death ².

In the 80's, defects in energy metabolism in ND have been observed *in vivo* using positron emission tomography (PET) to measure loco-regional consumption of glucose using the positron emitter [¹⁸F]-fluorodeoxyglucose (FDG). The most striking reductions were found in the striatum of HD patients and in some areas of the cerebral cortex in AD patients (i.e. parieto-temporal, frontal, and posterior cingulate cortices, see ³ for references). These metabolic alterations have often been interpreted to be a consequence of reduced synaptic activity and/or the loss of neurons.

There is also evidence that ND are associated with impaired mitochondrial function. Many biochemical observations in post mortem brains have suggested that respiratory chain complexes are differentially affected in the brains of patients depending on the disease and its stage of progression. These results may be biased by technical limitations (e.g. post-mortem delay), but they indicate that mitochondria may be affected early in ND. Complex I has been found to be affected in the substantia nigra in PD ⁴⁻⁶. This is compatible with the fact that mitochondrial toxins that target complex I (MPP+, the active metabolite of MPTP, and the pesticide rotenone) can produce parkinsonism in humans and laboratory animals ^{7,8}. In HD, preferential defects in complex II (succinate dehydrogenase, and to a lesser extent, complex IV) have been reported in the striatum in symptomatic patients (for review see ⁹), and chronic administration of the complex II inhibitor, 3-nitropropionic acid, in rats and non-human primates produces neurodegeneration and symptoms that are similar to those of the human

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3 disease¹⁰⁻¹². Preferential defects of complex IV have been reported in the cerebral cortex in AD¹³⁻¹⁵.
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5 These observations have likely fueled the interest in the “mitochondrial” hypothesis for ND.

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7 Research related to the “mitochondrial” hypothesis also benefited from the steadily increasing
8
9 knowledge of mitochondrial physiology over the past decades and the direct implication of this
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11 organelle in neurological disorders. Indeed, point mutations or small mitochondrial DNA deletions
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13 cause complex diseases with neurological signs (for review see¹⁶). In the early 90’s, the “apoptosis”
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15 era fueled the discovery of crucial mitochondrial functions in the control of cell survival¹⁷⁻²¹.
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17 Surprisingly, this research led to the observation that, in some cases, cell death actually requires
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19 energy. The apoptosome (Apaf1-caspase9-Cytochrome C) is active if ATP levels are sufficient²².
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21 However, the triggering of apoptotic pathways disturbs the mitochondrial membrane potential and
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23 reduces the proton force. The release of cytochrome c eventually abrogates electron flow in the
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25 respiratory chain and increases the production of reactive oxygen species (ROS), further supporting
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27 the idea that mitochondria and energy defects may be center stage in ND²³.

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29 The fact that the risk of developing ND increases with age also provides circumstantial evidence for a
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31 possible role of mitochondria. Indeed, normal aging is associated with mitochondrial dysfunction and
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33 oxidative stress (for extensive review, see²⁴). One of the most convincing arguments is that there is a
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35 progressive, age-dependent accumulation of molecular markers of oxidative stress in mitochondrial
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37 and nuclear DNA in the human brain, leading to relatively selective defects in the expression of genes
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39 related to energy metabolism²⁵. These changes (along with others that are linked to proteostasis,
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41 DNA repair, and autophagy) may render neurons and astrocytes particularly vulnerable to
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43 pathological processes in the ageing brain²⁶.

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45 Recent data from human genetics indicate that, in many cases, ND of genetic origin (i.e. familial) are
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47 caused by mutations in genes encoding proteins localized in mitochondria and/or proteins in which
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49 the mutations indirectly control mitochondrial functions. This is particularly true for PD, in which key
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51 genes encode mitochondrial proteins such as DJ-1, PINK1, and Parkin. These proteins play a key role
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53 in quality control of mitochondria in cells through regulation of a mechanism called mitophagy,
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55 where damaged/dysfunctional mitochondria are eliminated, to avoid further cellular damage^{27, 28}. In
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57 HD, mutant huntingtin interacts with mitochondria, reduces their capacity to cope with Ca²⁺ overload
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59 ²⁹, alters the fission-fusion equilibrium of mitochondria³⁰, and possibly their biogenesis,^{31, 32}. Master
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61 genes that regulate mitochondrial homeostasis/biogenesis/oxidative stress, and more generally,
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63 metabolism, such as PGC-1a, AMPK, Tfam, Nrf2, SIRT1 and SIRT3 may play a role in HD, PD, and AD³³.

Cellular specificity of energy metabolism

The brain has higher energy needs than other organs and almost no energy reserve. This is probably why it is so vulnerable to even subtle deficits of both nutrient and oxygen supply. Glucose is the main energy substrate for the adult brain and is mostly metabolized via the TCA coupled to oxidative phosphorylation in mitochondria. An important issue that has not yet reached a consensus concerns the respective contribution of the cellular processes performed by the major cell types of the brain (neurons and glia) to the overall energy budget. Theoretical work, mostly performed by the group of David Attwell³⁴⁻³⁶ has suggested that the pumping out of ions by the neuronal sodium-potassium pump to generate synaptic and action potentials, is responsible for the main energetic cost in the brain. The precise contribution of non-signaling tasks to the brain energy budget must be determined³⁷ before we can obtain a clear picture of the global brain energy budget, even if energy-efficient synaptic neurotransmission dominates. Although the greatest portion of energy expenditure is attributed to neuronal activity, the contribution of glial cells is non-negligible. Data obtained using many different experimental approaches have provided a comprehensive view of how the regulation of energy metabolism is compartmentalized in the brain. This is of great importance because it allows the targeting of specific cells and energy metabolism pathways in the context of energy deficits that occur during ND. The distinctive metabolic profiles of glial cells have been recently reviewed³⁸ and only some recent key points will be summarized here. ATP production is derived from two types of processes: oxidative phosphorylation, an electrochemical process occurring within the mitochondria, and glycolysis, a chemical process that takes place within the cytosol. A transcriptomic study was recently performed in acutely isolated and FACS-purified astrocytes and neurons followed by RNA sequencing. It provides compelling evidence that the expression pattern of selected genes involved in glucose metabolism confers astrocytes with the ability to dynamically upregulate glycolysis, whereas neurons cannot³⁹. These astrocyte-specific genes encode the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (Pfkfb3), a key positive modulator of glycolysis; the isoform PKM2 of the enzyme pyruvate kinase (PK), that regulates the formation of pyruvate; the isoform LDHB of the lactate dehydrogenase, that catalyzes the conversion of pyruvate into lactate, and pyruvate dehydrogenase kinase (PDK4) that inactivates pyruvate dehydrogenase. The absence of the expression of this set of genes in neurons explains why they may not be able to upregulate glycolysis when needed, in particular, when stressed. Instead, neurons use glucose to maintain their antioxidant status via the pentose phosphate pathway (PPP). Together with other NADPH regenerating systems, such as NADP-dependent isocitrate dehydrogenase and malic enzyme (ME), the PPP is one of the main producer of reducing equivalents, which are used to regenerate reduced glutathione⁴⁰. It is worth mentioning that oxidative metabolism may also increase in

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3 astrocytes during period of intense and prolonged functional activation ⁴¹. Therefore, astrocytes and
4 neurons use two complementary metabolic pathways to produce energy from glucose. Neurons
5 must have access to another oxidative substrate, since most of the energy used for signaling appears
6 to be consumed at the synapses and they cannot increase the production of pyruvate to face higher
7 energy demands during increased synaptic activity. Compelling evidence accumulated over two
8 decades suggests that excitatory activity-dependent lactate formation and release by astrocytes may
9 provide these additional oxidative energy substrates for neurons (see ⁴² for original contribution). In
10 addition to glucose oxidation and aerobic glycolysis, creatine/phosphocreatine (Cr/PCr) metabolism
11 may also be essential for the storing and buffering of high phosphate-bound energy in neural tissue.
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20 *Neuron-Astrocyte Metabolic Coupling*

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22 This shuttling of lactate, known as the Astrocyte-Neuron Lactate Shuttle (ANLS) hypothesis, provides
23 a functional explanation for the existence of the cellular compartmentalization of glycolysis and
24 oxidative phosphorylation. It describes several key molecular steps by which the brain can finely
25 orchestrate the delivery and use of the energy substrates necessary to sustain highly metabolically
26 demanding pre- and postsynaptic processes. It is not our purpose to review the debate that this
27 hypothesis has raised during almost 20 years ^{43, 44}. Data obtained *in vivo*, including ours ⁴⁵⁻⁴⁷ has
28 shown that the transport of synaptically-released glutamate into astrocytes promotes glucose uptake
29 and lactate release by a mechanism that involves an increase in intracellular sodium (see ³⁸ for a
30 comprehensive review). Data recently obtained in *Drosophila* confirm the existence of these
31 metabolic fluxes between glia and neurons and suggest that the underlying division of labor is likely
32 an ancestral feature of nervous system design ⁴⁸.
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41 More relevant in the context of ND, the ANLS model led to the identification of molecular actors,
42 located in specific cell types, that play an important role in regulating the signaling pathways of this
43 complex metabolic cell-to-cell trafficking (Figure 1). Among these proteins, glutamate (GLAST and
44 GLT-1) and monocarboxylate transporters (MCTs, responsible for lactate shuttling) have been well-
45 studied ^{49, 50}. Some of these proteins are specifically located in astrocytes, suggesting that astrocytic
46 metabolic dysfunction induced by mutations or functional alterations of these proteins, may also
47 contribute to neurodegenerative processes. The notion that cell subtypes other than neurons may
48 serve distinct and necessary roles in neurodegeneration is known as non-cell-autonomous toxicity ⁵¹,
49 and may be particularly relevant for metabolic energy coupling.
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Aerobic glycolysis: other purposes beyond ATP production

What may be less well-appreciated outside of the cancer field, is the fact that, although ATP generated from aerobic glycolysis is undoubtedly important for cellular function, the importance of aerobic glycolysis extends beyond rapid ATP production to allow nutrient assimilation into biosynthetic precursors and facilitate biomass accumulation⁵². It is well accepted that aerobic glycolysis is the dominant metabolic pathway during development for supporting cell proliferation. However, the notion that aerobic glycolysis also plays an important role during early postnatal development, when lipids and proteins are needed for the processes of axonal elongation and synaptogenesis, and in adulthood to support the molecular modifications that underlie plasticity of dendritic spines and synapses^{53, 54}, is still a matter of debate. In addition to supporting nucleotide (purines and pyrimidines) biosynthesis, glycolysis is a source of carbon for lipid precursors, phospholipids and amino acids. These molecules are all required for fundamental processes such as nucleic acid assembly, myelination, axonal elongation and synaptogenesis. A low-flux pathway (biosynthesis) branching from a high-flux pathway (glycolysis) is probably highly sensitive to decreases of the glycolytic flux, even if the utilization rate of glycolytic intermediates for the biosynthesis of macromolecules accounts for less than 10% of the glycolytic rate. Thus, any slowing of the glycolytic flux may lead to reduced production of those glycolysis-dependent molecules, in addition to decreasing ATP production. Such a shortage in the synthesis of these molecules may potentially contribute to the pathogenesis of ND. Two, lactate and D-serine, merit specific attention in the context of ND, that are often characterized by early synaptic dysfunction. These two molecules derived from aerobic glycolysis, are mostly produced by astrocytes (Figure 1), and are necessary for the maintenance of long-term potentiation (LTP), a long-lasting increase in synaptic efficiency that underlies learning and memory⁵⁵⁻⁵⁷. Lactate is the end-product of aerobic glycolysis, whereas D-serine is also generated from glucose, but through diversion of the glycolytic intermediate, 3-phosphoglycerate (3PG) into the phosphorylated pathway. The first committed step in this pathway is the oxidation of 3PG to 3-phosphohydroxypyruvate by the astrocytic-specific enzyme, 3-phosphoglycerate dehydrogenase (PHGDH)⁵⁸. PHGDH plays a critical role in the direct generation of D-serine⁵⁹ in astrocytes and indirectly in neurons, after import of astrocytic L-serine⁶⁰. These data suggest the existence of astrocyte-to-neuron transfer of both lactate and L-serine/D-serine, necessary for critical physiological functions, such as plasticity and memory, which are specifically impaired in ND such as AD.

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3 *The role of oligodendrocytes and Cr/PCr metabolism in brain energy homeostasis*
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6 To date, most studies on brain bioenergetics have investigated how ATP is produced from either the
7 oxidation of glucose and downstream metabolites, a pathway that predominates in neurons, or
8 aerobic glycolysis, a mechanism mainly used by glial cells ³⁸. As discussed, a specific feature of the
9 organization of brain energetics is the tight metabolic coupling between neuronal and glial cells that
10 was first illustrated by the glutamate-glutamine(-GABA) shuttle between neurons and astrocytes
11 more than 30 years ago: the selective expression of pyruvate carboxylase and glutamine synthase by
12 astrocytes is essential for the recycling of glutamate released from neurons in glutamatergic
13 neurotransmission. More recently, a tri-cellular compartmentation of brain energy metabolism
14 between neurons, astrocytes and oligodendrocytes has been proposed (Figure 1) via the lactate
15 shuttle involving astrocytes at synapses and oligodendrocytes at axons ⁶¹. Tri-cellular
16 compartmentation has also been suggested for the metabolism of *N*-acetylaspartate (NAA) ⁶².
17 However, several issues related to the role of oligodendrocytes in brain bioenergetics remain
18 unresolved. The observation of high levels of creatine synthesis enzymes in oligodendrocytes shed
19 light on a novel neuron-glial relationship for brain energy homeostasis. Indeed, the Cr/PCr shuttle, as
20 reversibly catalyzed by Cr kinases (CKs), may be essential for the storing and buffering of high
21 phosphate-bound energy in tissues with high-energy demands. Creatine biosynthesis from arginine
22 and glycine is carried out by two sequential steps catalyzed by L-arginine:glycine amidinotransferase
23 (AGAT) and S-adenosylmethionine:guanidinoacetate N-methyltransferase (GAMT). In the brain, the
24 phosphate transfer reaction between Cr and PCr is reversibly catalyzed by CKs present in the
25 mitochondria (uMt-CK) and cytoplasm (B-CK). Cells with the ability to synthesize Cr generally tend to
26 have a low-functioning PCr/CK system. Similarly, the *de novo* synthesis of Cr takes place in glial cells,
27 reflected by the predominant expression of AGAT and GAMT in oligodendrocytes and astrocytes.
28 Conversely, the Cl⁻-dependent creatine transporter (CrT) is expressed predominantly in neurons and
29 oligodendrocytes ^{63, 64}. The phosphotransfer reaction is also characterized by opposing expression
30 patterns, as uMt-CK is expressed exclusively in neurons whereas B-CK is expressed in astrocytes with
31 lower selective expression in inhibitory neurons – e.g. striatal medium spiny neurons ⁶⁵⁻⁶⁷. Of note,
32 the neuronal expression of CrT closely coincides with the ubiquitous neuronal expression of uMt-CK.
33 Thus, signaling and other energy-intensive properties of neurons may be supported by the specific
34 expression of the CK that is linked to oxidative metabolism (i.e., uMt-CK) along with a higher capacity
35 for Cr uptake through high CrT expression. In contrast, glial cells, that have lower energy
36 requirements, appear to require lower levels of Cr and utilize the glycolysis-linked B-CK. It is possible
37 that axonal creatine is supplied, at least partially, from local oligodendrocytes that enwrap axons,
38 because axons consume a considerable amount of metabolic energy to maintain the ionic gradient
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3 across the axolemma, propagate action potentials, and transport molecules and organelles.
4 Accordingly, the segregation between Cr producers and Cr users emphasizes the tight metabolic
5 coupling between neurons, astrocytes and oligodendrocytes, and the dysregulation of Cr/PCr
6 metabolism may contribute to the pathophysiology of chronic CNS diseases. We used microwave
7 fixation systems, which instantly inactivate brain enzymes and conserve concentrations of high-
8 energy phosphates (ATP, PCr) while preserving the structure of the brain, in HD models and we
9 showed that the chronic energy failure characterized by ATP depletion in the striatum of HD mice
10 was preceded by an increased pool of Cr and PCr in motor asymptomatic mice. This increase in the
11 Cr/PCr pool was not explained by increased expression of AGAT, GAMT, or CrT, suggesting rather an
12 alteration of the phosphotransfer reaction mediated by CK⁶⁸.
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22 **Novel methods to assess energy metabolism in the brain**

23 The number of methods to assess energy metabolism in patients is extremely limited, given the
24 complexity of existing metabolic pathways and their cellular and subcellular compartmentalization.
25 Existing clinical techniques focus on only highly concentrated brain metabolites, because of their
26 limited sensitivity (in space and time), and are blind to most cellular processes (see Table 1). Thus,
27 our vision of energy changes in ND is likely too simplistic. We need to improve our understanding of
28 brain energy metabolism in health and disease through experiments conducted at different scales in
29 cell and animal models to implement new methods in the clinical setting in the long term. Novel
30 approaches have recently emerged that may better address the questions of compartmentalization,
31 cell specificity, and the dynamics of energy metabolism in ND. Some methods are relatively invasive
32 (in particular, those using fluorescence) and can be used only in animal models or cultured cells, but
33 other approaches, based on nuclear magnetic resonance (NMR) and Positron emission tomography
34 (PET), can be used on human subjects. These various approaches are complementary and may help
35 to better characterize the role of energy metabolism in ND.
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45 It is not our purpose to comprehensively list all the methods and studies that have been carried out
46 to probe brain metabolism, but rather to offer a brief overview of the broad spectrum of approaches
47 that have emerged in recent years that may provide significant insight into energy metabolism in ND
48 in the near future.
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51 *Biosensors to probe cellular energy metabolism*

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56 There is no simple way to concomitantly investigate different facets of energy metabolism. However,
57 different fluorescent probes have been developed to study key metabolic fluxes in different brain cell
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3 types. Fluorescent probes offer several advantages, including high resolution in space and time,
4 allowing the study of time-dependent phenomena at the subcellular scale. The general principle is
5 based on the selective dependence of the fluorescence level of the probe on the binding of a
6 molecule of interest such as glucose, lactate and ATP (for a review see ⁶⁹). In some cases, the binding
7 of the molecule directly affects the fluorescent signal of the reporter protein for a given excitation
8 wavelength. In others, the probes are engineered proteins with two fluorophores (variants of GFP)
9 separated by a peptide domain in which the molecule of interest binds and changes the
10 conformation of the protein, bringing the two fluorophores closer together. The two fluorophores
11 are chosen to allow detection of a FRET signal (i.e. excitation/emission wavelengths of each allow for
12 intra-molecular “Föster resonance energy transfer” – photons emitted by the donor fluorophore
13 excite the acceptor fluorophore): the closer the two fluorophores, the higher the FRET signal. Thus,
14 the FRET signal directly depends on the amount of the molecule of interest that binds the sensor.
15 The improvement of fluorescent probe-based methods also arises from microscopy devices and
16 methods to study the proteins (two photon imaging, confocal microscopy, fluorescence-lifetime
17 imaging microscopy). These methods have great potential to reveal key aspects of energy
18 metabolism in cells, tissue slices, and *in vivo* in small animals; a few very promising examples are
19 described below.
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31 Energy production requires the entry of glucose into brain cells (neurons and astrocytes) and its
32 catabolism through glycolysis and the TCA cycle, or via alternative pathways, such as the pentose
33 pathway. The development of glucose sensitive fluorescent probes has made it possible to measure
34 the consumption of glucose at the cellular scale (either in neurons or astrocytes), i.e. not only its
35 intracellular concentration, but its rate of disappearance when the entry flux is null. This can be
36 carried out in transfected cells and, more recently, in tissue slices, and *in vivo*, through the use of
37 viral vectors to transduce neurons (and/or astrocytes). This approach confirmed that, in addition to
38 glutamate, small increases in extracellular K⁺ levels can also trigger aerobic glycolysis in astrocytes ⁷⁰,
39 an effect mediated by the electrogenic Na⁺/HCO₃⁻ co-transporter, NBCe1 ⁷¹.
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47 The recent development of the FRET sensors, Laconic (for lactate) and Pyronic (for pyruvate), allowed
48 the discovery that astrocytes possess a lactate channel ⁷². The expression of these sensors in neurons
49 or astrocytes via adeno-associated viral vectors (AAVs), in combination with *in vivo* two-photon laser
50 scanning microscopy, recently provided data that supports the hypothesis of a lactate gradient from
51 astrocytes to neurons ⁷³, which was indirectly suggested many years ago ⁴².
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55 We have started using those FRET sensors in ND models to precisely dissect out whether alterations
56 of glucose consumption (often reduced in ND models and patients, based on PET studies, see below)
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3 occur in neurons and/or astrocytes ⁷⁴. It may also be particularly informative to determine whether,
4 and how, the lactate shuttle between astrocytes and neurons is affected in animal models of ND.
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7 Many other metabolic FRET sensors have been designed ⁷⁵, including Ataems and PercevalHR to
8 monitor ATP levels and its rate of synthesis, a key parameter for determining the status of energy
9 metabolism. Other sensors have been recently developed to measure the levels of different
10 metabolites including citrate, an important regulatory molecule for the control of glycolysis ⁷⁶, and
11 pyruvate ⁷⁷. We are still far from being able to mount a comprehensive study of the metabolic
12 network and are still lacking FRET sensors to probe metabolites involved in glycogen synthesis and
13 degradation, the pentose phosphate pathway and Cr/PCr metabolism. It will be necessary to further
14 improve their sensitivity, specificity, and independence of pH changes, and limit their potential
15 cellular toxicity.
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22 Biosensors can be specifically targeted to specific organelles, such as the mitochondria, by fusion to
23 targeting sequences, since they are genetically encoded. Molecules involved in energy production
24 (ATP, NADH, pH), ROS (superoxide, hydrogen peroxide), the redox state, and second messengers
25 (cAMP, Ca²⁺) have been studied in the mitochondria. Fluorescent dyes able to measure the
26 mitochondrial membrane potential ⁷⁸ are commonly used to monitor changes in this important
27 physiological mitochondrial parameter as it relates to the cell's capacity to generate ATP by oxidative
28 phosphorylation. Membrane potential itself plays a key role in regulating respiratory chain activity
29 and in coupling the extrusion of protons to generate the protonic force necessary for the synthesis of
30 ATP from ADP and Pi by F1/F0 ATPase.
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38 These tools are highly promising for elucidating the potential dysfunctions of energy metabolism
39 fluxes in cellular and, in some cases, animal models of ND, but they obviously cannot be used in
40 patients.
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43 44 45 *Non-invasive in vivo imaging / neurochemistry*

46 47 48 1-Positron emission tomography (PET)

49 [18F]-FDG is still a universal marker of energy metabolism with relatively disease-specific uptake
50 reduction patterns. Bilateral temporo-parietal areas are mainly affected in AD, whereas either the
51 frontal or the temporal regions exhibit [18F]-FDG uptake reductions in fronto-temporal lobar
52 degeneration. [18F]-FDG also allows discrimination between primary PD and atypical parkinsonian
53 syndromes, as major glucose consumption deficits are only found in the latter (see ⁷⁹ for references).
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60 Reduced glucose consumption in the caudate/putamen is also reported in the brain of HD patients ⁸⁰.

Probably more relevant, this reduction in striatal glucose consumption is often seen in presymptomatic gene carriers. However, [^{18}F]-FDG cannot distinguish the fate of a glucose molecule. PET studies that utilize glucose uptake indiscriminately follow glucose utilized by oxidative phosphorylation and aerobic glycolysis. Caution is required when interpreting data from radiolabeled glucose PET scans without complementary radiolabeled oxygen data, given the presence of aerobic glycolysis in the brain during postnatal neurodevelopment and adulthood. When measurements of the molar ratio of cerebral oxygen metabolism to cerebral glucose metabolism ($\text{CMRO}_2/\text{CMRglc}$) were performed, a higher ratio was found in the striatum of HD patients than in healthy controls, with the CMRO_2 unchanged, and a lower CMRglc . These data are consistent with a selective defect of glycolysis in the early HD striatum and not defective mitochondrial oxidative phosphorylation⁸¹. These data also suggest that astrocyte dysfunction may be involved in the pathogenesis of HD since glucose is preferentially processed via glycolysis in astrocytes. The molar ratio of oxygen consumption to glucose utilization, also known as the oxygen–glucose index (OGI), is not equal to six in the healthy brain, which would be expected if all the metabolized glucose was converted into carbon dioxide and water. This means that a fraction of the glucose is used in aerobic glycolysis, despite the presence of sufficient oxygen⁸². In addition, there are striking regional variations in aerobic glycolysis in the normal human brain – significantly higher in medial and lateral parietal and prefrontal cortices than in other regions – even if this has been recently challenged⁸³. Strikingly, a spatial correlation was recently found between aerobic glycolysis in the brain and amyloid- β ($\text{A}\beta$) deposition⁸⁴, suggesting a possible link between regional aerobic glycolysis and the development of AD pathology. Probing the metabolic fate of glucose in more detail may be important for understanding the pathophysiology of many NDs.

2-MRI methods

2.1. Principles of MRI

Magnetic resonance imaging (MRI) relies on the detection of resonance signals generated by the nuclei of hydrogen atoms (^1H), sometimes simply called “protons”, of water molecules when submitted to radiofrequency stimulation in a static magnetic field. MRI is mostly known for its capacity to non-invasively provide high resolution images of brain structures, and its application to ND has largely contributed to a better understanding of the morphometric changes in cohorts of patients affected by these disorders, for example striatal atrophy in HD or hippocampal atrophy in AD. Advanced diffusion-weighted MRI techniques, which are sensitive to the effect of tissue microstructure on water diffusion, can also provide maps of water diffusion properties and even maps of structural connectivity, i.e. images of white matter fibres reconstructed from diffusion

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3 anisotropy – e.g. see ⁸⁵ for recent review including applications to ND. Some structural information at
4 a micrometric scale (well below the size of a single pixel, which is typically in the 100 μm to 1 mm
5 range) can also be estimated from water diffusion, such as cellular density, the extracellular volume
6 fraction, or axon diameter ⁸⁶. Furthermore, MRI can be exploited to provide not only structural
7 information, but also functional measures.
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10 11 12 *2.2. Principles of functional MRI*

13 MRI can be rendered sensitive to local (intra-pixel) magnetic field inhomogeneity using a gradient
14 echo sequence. Using such a sequence, if the magnetic field is inhomogeneous within a given pixel,
15 magnetization clusters experiencing different values of the magnetic field will be progressively
16 dephased relative to each other (the resonance frequency being proportional to the magnetic field),
17 ultimately leading to a reduced average magnetisation in the pixel, and thus a reduced signal. This
18 principle has been applied to develop the most common modality of functional MRI (fMRI), where
19 the so-called Blood Oxygenation Level Dependant (BOLD) effect on magnetic field inhomogeneity is
20 detected during brain activity. The BOLD effect relies on the local transient increase of the
21 oxyhemoglobin-to-deoxyhemoglobin ratio during the haemodynamic response associated with
22 neuronal activity. Since deoxyhemoglobin induces magnetic inhomogeneity, activated regions of the
23 brain, with less deoxyhemoglobin, exhibit stronger signals in gradient echo images than in the resting
24 condition ⁸⁷. Functional MRI is either used with task-based paradigms, in which the mean of the
25 baseline state is subtracted from the mean of the stimulated state to identify activated regions
26 associated with the task, or it is used to study the brain at rest, in the absence of an explicit task
27 (resting state BOLD-fMRI). Other fMRI modalities have been proposed, but are much less common
28 than BOLD fMRI. They include arterial spin labelling (ASL) to measure variations in tissue perfusion in
29 each pixel ⁸⁸, which can help to untangle confounding mechanisms, leading to variations of the BOLD
30 effect, and diffusion-weighted MRI, to presumably detect cellular swelling during activation ⁸⁹. These
31 fMRI techniques are very promising for the detection of changes in neurovascular modifications
32 linked to ND, both in preclinical and clinical studies. However, the mechanisms underlying the
33 changes seen with these methods cannot be directly assessed. A reduction in the BOLD signal and/or
34 perfusion can result from a reduced neuronal activation, abnormal neuron/astrocyte coupling, a
35 reduced vascular response, or a combination of all these components of the neurovascular response.
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51 52 53 *2.3. Chemical Exchange Saturation Transfer (CEST) MRI*

54 A new MRI modality called CEST for Chemical Exchange Saturation Transfer has been proposed to
55 image some metabolites with good spatial resolution ^{90,91}. This technique exploits labile protons such
56 as amine ($-\text{NH}_2$), amide ($-\text{NH}$), or hydroxyl ($-\text{OH}$) groups which are in exchange with free water. Thus,
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3 labile protons exhibit a resonance frequency that is shifted relative to the bulk proton frequency.
4 Exchangeable protons can also be selectively saturated using radiofrequency (RF) impulsion, leading
5 to a decrease of the water signal due to magnetization exchange. The indirect detection of less
6 concentrated protons through the observation of those that are highly concentrated confers high
7 sensitivity to CEST imaging, making possible *in vivo* mapping of several metabolites with good spatial
8 resolution. By optimizing saturation parameters of the RF impulsion (e.g. saturation intensity, offset
9 and duration), it is possible to probe different exchanging protons. CEST offers the possibility to
10 detect metabolites such as glutamate ⁹¹, glucose ^{92, 93} and *myo*-inositol ⁹⁴. These endogenous
11 molecules can be used to explore energy metabolism in ND as non-invasive biomarkers, as they are
12 involved in many biological pathways.
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20 Glutamate is probably amongst the best candidates of the metabolites detectable by CEST imaging as
21 it is one of the most highly concentrated (approximately 10 mmol/kg) and the exchange properties of
22 its amine function result in good detection in high magnetic fields ($\geq 7T$) ⁹¹. Glutamate plays a central
23 role in brain energy metabolism as it is directly linked to the Krebs cycle though its equilibrium with
24 alpha-ketoglutarate ⁹⁵. Consequently, modifications of glutamate concentration may indicate
25 changes in energy metabolism and/or compensatory mechanisms to maintain homeostasis ^{96, 97}. In
26 addition, changes in glutamate levels may be indicative of a disturbance of the glutamate/glutamine
27 cycle, which has a central role in energy homeostasis between astrocytes and neurons. GluCEST
28 imaging has been used in several rodent ND models, such as AD ^{91, 98} and HD ⁹⁹. In these studies, the
29 decrease of gluCEST contrast was consistent with decreased metabolic pools of glutamate as
30 measured by magnetic resonance spectroscopy (MRS). However, gluCEST offers the opportunity to
31 detect alterations in glutamate levels in very thin structures, such as the corpus callosum, which
32 would not have been achievable using MRS, due to its better spatial resolution. The decrease of
33 glutamate concentration was accompanied by a decrease of NAA, another metabolite assumed to be
34 mostly located in the neuronal compartment and considered to be a good neuronal marker ^{100, 101}.
35 Thus, the decrease in gluCEST contrast suggested a potential alteration of neuron integrity or
36 function. The high resolution of gluCEST has also been exploited in a rat model of astrocyte reactivity,
37 using a lentiviral vector encoding the ciliary neurotrophic factor (CNTF) ¹⁰². Induction of CNTF
38 expression through lentiviral gene transfer in the rat striatum significantly decreased the levels of
39 neuronal metabolites, suggesting remodeling of the striatal metabolism or the occurrence of
40 compensatory mechanisms. It has also been shown that the brain region exhibiting low gluCEST
41 contrast closely matched the region containing reactive astrocytes (using vimentin staining). This
42 demonstrated that gluCEST may also serve as a potential biomarker of astrocyte reactivity and a tool
43 to monitor metabolism adaptations. The potential of gluCEST imaging in clinical studies has already
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3 been demonstrated in healthy volunteers^{91, 103, 104} and in patients with temporal lobe epilepsy¹⁰⁵.
4 However, the fast exchange regime of amine protons requires high magnetic fields (≥ 7 T) to fulfill
5 the required CEST conditions⁹¹, but the emergence of clinical 7T scanners should ease the transfer of
6 gluCEST imaging for clinical applications.
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10 Another important application of CEST imaging is the imaging of hydroxyl protons of *myo*-inositol,
11 called miCEST⁹⁴. Exchange parameters and chemical shifts of -OH protons are different than those of
12 -NH₂ protons of glutamate. Thus, it is possible to specifically probe these protons by optimizing the
13 saturation module of the CEST sequence. *Myo*-inositol is mostly located in glial cells and is a good
14 marker of cellular proliferation. The concentration of *myo*-inositol may be altered in ND, in particular
15 in the brains of AD patients¹⁰⁶. Consequently, miCEST could be used to image changes in *myo*-
16 inositol levels throughout the brain and serve as a glial cell marker.
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22 Finally, several studies have proposed to image energy metabolism *in vivo* through CEST imaging of
23 glucose, called gluCEST⁹². The gold-standard for the detection of energy defects is PET, which
24 requires a radio-labeled analog of glucose (¹⁸F-FDG). However, access to PET-scan is limited relative
25 to MRI and the use of radioactive agents may limit the number of exams. The possibility to use an
26 MRI scanner and natural agents, such as glucose, may open new opportunities for mapping glucose
27 metabolism *in vivo* with many potential applications for the investigation of ND.
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33 Several issues need to be taken into account in order to properly analyze CEST data. Even if
34 saturation parameters can be optimized to specifically probe one metabolite, one cannot exclude
35 that the CEST contrast can be contaminated by signal arising from other brain metabolites or mobile
36 protons linked to macromolecules. This issue has already been investigated for the gluCEST contrast
37 at 7T⁹¹. Thanks to a phantom containing metabolites at their physiological pH and concentration and
38 simulation of theoretical CEST effects for each metabolite, authors estimated that glutamate
39 contributed to about 70-75% to the total gluCEST contrast. Although not purely glutamate-
40 dependent, gluCEST contrast provide a good measurement of glutamate distribution. Concerning
41 metabolites with hydroxyl groups, such as *myo*-inositol or glucose, the contamination of confounding
42 effects is probably more important as the resonance frequency of -OH group is closer to the water
43 frequency (about 1 ppm). In addition to the concentration, the CEST contrast is also dependent on
44 the proton exchange rate. This parameter is sensitive to physiological parameters such as
45 temperature or pH. If we can reasonably consider that temperature is rather constant and has a
46 minor impact, pH can dramatically modify CEST contrast as illustrated with the increase of gluCEST
47 contrast in a rat model of stroke due to pH decrease⁹¹. In order to address this potential confounding
48 effect, it is necessary to acquire CEST contrasts for different saturation offsets (this is called a z-
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spectrum). Interestingly, the shape of the z-spectrum changes with the pH whereas CEST effect varies linearly with the concentration⁹¹. Consequently, acquisition of a z-spectrum (and not only a single CEST acquisition) can help isolating both contributions.

With the use of higher magnetic fields (>7T), one can anticipate a higher specificity of the CEST contrast thanks to a better selectivity of the saturation module. Also, modeling approaches of proton exchange processes are under evaluation in order to decouple specific contribution of each metabolite *in vivo*^{91,107}.

The versatility of CEST imaging to probe several metabolites offers the possibility to monitor the status of different cell types (neurons by glutamate and glial cells by *myo*-inositol) and to image the consumption of energy substrates, such as glucose. This confers to CEST the potential to provide valuable clues about potential energy defects and to image various pathological features in a single experiment (Figure 2).

3. Magnetic resonance spectroscopy (³¹P, ¹H, ¹³C)

3.1. Principle of MRS

The MRI signal originates from the ¹H nuclei of water molecules, but many other nuclei in other molecules can also be detected by magnetic resonance. This is the basis for MRS, which allows separating and quantifying various metabolites non-invasively. Although MRS requires larger detection volumes than MRI, due to the lower abundance of metabolites relative to water, it offers an unparalleled view inside metabolic processes *in vivo*. For a detailed introduction about *in vivo* MRS principles and methodology, we refer the reader to¹⁰⁸. Very briefly, the principle is the following: nuclei are surrounded by electrons which partially shield them from the external magnetic field B₀ of the magnetic resonance scanner, thus leading to very small variations of the magnetic field experienced by these nuclei. These variations in turn result in small shifts (the so called “chemical shift”, generally expressed in parts per millions or ppm) of the magnetic resonance frequency of nuclei having non-zero spins. Hence, depending on its chemical structure, each molecule can be characterized by a specific spectrum, i.e. a resonance pattern at different chemical shifts, the amplitude of the resonance pattern being related to the concentration of the corresponding molecule.

3.2. ¹H NMR spectroscopy

Proton (¹H) MRS is the most common modality of MRS, because it yields the highest detection sensitivity (due to the abundance of ¹H nuclei in biological tissues and fluids) and requires no specific hardware outside of that of standard MRI. However, the chemical shift dispersion of metabolites in ¹H MRS is relatively narrow, making many resonances overlap. This complicates spectral

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3 quantification, which can be advantageously performed using linear decomposition. With
4 appropriate acquisition and analysis, it is possible to quantify metabolites primarily associated with
5 neurons, in particular NAA and glutamate (Figure 2), as well as metabolites thought rather to be
6 associated with astrocytes¹⁰⁹ such as choline, *myo*-inositol, and even glutamine, which can be
7 separated from glutamate in a high magnetic field, or associated with oligodendrocytes such as
8 creatine. It is relatively standard procedure to normalize the metabolite signal relative to that of total
9 creatine, which is assumed to remain relatively stable (around 8-10 mM), at least in the healthy
10 brain. This is however debatable, in particular in a pathological context, and it is instead possible to
11 perform absolute quantification, e.g. relative to the water signal for which the concentration can be
12 estimated from the voxel composition¹¹⁰.

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20 Levels of NAA and *myo*-inositol are considered to be potential biomarkers of brain dysfunction in AD
21 and PD subjects, based on numerous clinical studies^{111 112}. MRS studies of HD patients have also
22 shown a consistent reduction in NAA levels^{113, 114}, suggesting neuronal stress and possibly
23 mitochondrial changes, since NAA is largely synthesized in mitochondria in neurons. Studies using
24 MRS have also identified increased glutamate/glutamine and *myo*-inositol peaks, suggesting a
25 metabolic imbalance between neurons and astrocytes in HD^{114,115}.

31 3.3. ³¹P NMR spectroscopy

32 Phosphorus (³¹P), with natural abundance of 100%, is NMR visible, such that all phosphorylated
33 metabolites can be observed using ³¹P MRS, which is of course of particular interest for energy
34 metabolism. The most prominent peak is phosphocreatine (PCr). ATP can also be quantified, but the
35 concentrations of ADP and AMP are too low for them to be detected (Figure 2). The small peak of
36 inorganic phosphate Pi is also visible, but its quantification remains relatively difficult. These
37 measured concentrations are often relative, as it is difficult to have an internal ³¹P reference of
38 known concentration. Measuring the difference in the chemical shift between the Pi and PCr peaks
39 also yields an estimate of intracellular pH. ³¹P MRS requires a dedicated radiofrequency channel, and
40 is complicated by the relatively short T₂ transverse relaxation times and the 2.5-fold lower
41 gyromagnetic ratio relative to that of protons, which may result in relatively low detection sensitivity.
42 We used ³¹P MRS in HD patients and found that intracellular pH was increased in the brain¹¹⁶.
43 Another recent ³¹P MRS study reported increased Pi/PCr and Pi/ATP ratios during visual stimulation
44 in the occipital cortex (which reflects increased Pi and ADP levels to increase mitochondrial ATP
45 production under normal aerobic conditions) in controls, but not in HD, suggesting an impairment in
46 the adaptation to energy demand¹¹⁷. This abnormal response to activation was confirmed in a later
47 study, which also demonstrated that treatment with triheptanoin, an anaplerotic compound
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3 providing two key intermediates to the TCA cycle, restored the increased Pi/PCr ratio during visual
4 stimulation in HD patients at an early stage of the disease ¹¹⁸.

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7 Using ³¹P MRS, it is also possible to perform magnetization transfer experiments to measure steady-
8 state reaction rates for ATP synthase or creatine kinase (see ¹¹⁹ for review). Although this opens very
9 exciting possibilities for the study of energy metabolism, acquisitions remain long and difficult. This
10 probably explains why magnetization transfer ³¹P MRS remains the object of methodological
11 research and has been so far limited to a few studies in the healthy brain ¹²⁰⁻¹²⁵ and one study related
12 to schizophrenia ¹²⁶. However, such techniques would be of special interest to decipher the
13 mechanisms underlying the abnormal profiles of Cr/PCr metabolism that we have observed in both
14 HD patients and animal models ^{68,117}.

20 21 22 *3.4. Energy metabolism fluxes in neurons and astrocytes using ¹³C MRS*

23 *In vivo* carbon 13 (¹³C) MRS is a unique technique to study cellular metabolism in the brain and has
24 led to major advancements in the understanding of cerebral energy metabolism, neuron-astrocyte
25 compartmentalization and cooperation, and glutamatergic neurotransmission (see ¹²⁷; ¹²⁸ for recent
26 reviews). The basic principle is the following: ¹³C, the only stable isotope of carbon possessing a
27 nuclear magnetic moment, has a natural abundance of only ~1.1%, and is therefore practically
28 invisible, so that ¹³C MRS allows precise tracking of the metabolic fate of highly ¹³C-enriched
29 substrates by specifically identifying which metabolites are being labeled, and at what atomic
30 positions. Labeling patterns can provide information on the metabolic pathways involved, and
31 labeling dynamics contain quantitative information about metabolic fluxes. In most cases, glucose
32 labeled at position C1 ([1-¹³C]glucose) or C1 and C6 ([1,6-¹³C₂]glucose) is infused, leading to the
33 labeling of pyruvate at position C3. The labeled pyruvate enters the TCA cycle mostly via pyruvate
34 dehydrogenase and is then incorporated at position C4 of glutamate during the first turn of the cycle,
35 and at positions C2 and C3 of glutamate during the second turn. The label is exchanged between
36 glutamate and glutamine via the glutamate-glutamine cycle that occurs between neurons and
37 astrocytes (Figure 2). Due to specific metabolic features of neurons and astrocytes (presence of
38 pyruvate carboxylase exclusively in astrocytes, leading to the labeling of glutamate/glutamine in
39 position C2, and preferential compartmentation of glutamate in neurons and of glutamine in
40 astrocytes), the labeling patterns and dynamics may allow differentiating between both cell types.
41 Beyond the simple interpretation of ¹³C enrichment as quantified on spectra, adequate metabolic
42 modeling of these data allows, in theory, the separate quantification of pyruvate dehydrogenase
43 fluxes in neurons and astrocytes, as well as pyruvate carboxylase (in astrocytes), from which TCA
44 cycles can be derived, and the glutamate-glutamine cycle (see ¹²⁹ for review about metabolic
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3 modeling of *in vivo* data and ^{130, 131} for the latest developments and refinements). However, the
4 methodology requires the infusion of costly substrates over long periods (several hours), making it
5 very challenging to perform in humans, especially in patients. We refer the reader to ^{132, 133} for
6 reviews about ¹³C MRS in a clinical context, including some case reports of ¹³C MRS in
7 leukodystrophies and in poorly defined mitochondrial diseases, resulting in abnormal labeling
8 patterns, in particular the accumulation of labeled lactate, aspartate, and alanine. The same group
9 also performed a study in hepatic encephalopathy (a disease caused by chronic exposure to elevated
10 ammonia in the blood due to liver failure) in the human brain ¹³². Reduced labeling of glutamate at
11 position C2 and increased labeling of glutamine at position C2 were interpreted to be due to reduced
12 glucose oxidation and increased glutamate-glutamine cycling, using simple modeling. A study
13 performed in AD patients suggested a decrease of the neuronal TCA and glutamate-glutamine cycles
14 based on ¹³C enrichment, without modeling ¹³⁴. In all the above studies, no advanced metabolic
15 modeling was performed, so that the accurate quantification of metabolic fluxes, in particular the
16 specific quantification of neuronal and astrocytic TCA cycles, was not possible. A study performed
17 during the infusion of labeled glucose and labeled acetate (specific to astrocytes), combined with
18 metabolic modeling, showed the parallel decrease of the neuronal TCA and glutamate-glutamine
19 cycles and an increase of the astrocytic TCA cycle during healthy aging ¹³⁵. Note that other labeled
20 substrates can be infused to study their uptake and utilization by neurons and astrocytes, in
21 particular lactate ^{136, 137}. Although lactate and acetate metabolism have been shown to be altered
22 during hypoglycemia in type 1 diabetic patients ^{138, 139}, they have never been used in ND patients. In
23 the end, all these studies may be the prelude to the specific investigation of neuronal and astrocytic
24 metabolism and intercellular cycling in ND in humans even if the cost of ¹³C experiments may limit its
25 clinical development. Let's finally mention the possibility to hyperpolarize some labeled substrates
26 (such as pyruvate or lactate and even glucose ¹⁴⁰) before injecting them, leading to dramatically
27 increased signal-to-noise ratio (see ¹⁴¹ for review about principles and *in vivo* applications). Although
28 hyperpolarization typically disappears over a few dozen seconds due to relaxation, leaving little time
29 to label compounds lying far downstream on metabolic pathways, or derived from substrates via
30 very slow biochemical reactions, it can still bring valuable information about substrate consumption
31 and first metabolic steps, in particular lactate dehydrogenase activity. The redox state may also be
32 monitored *in vivo* using hyperpolarized ¹³C dehydroascorbate ¹⁴². How the improved spatial/temporal
33 resolutions of the measurement and the unique metabolic information gleaned through
34 hyperpolarized ¹³C might be useful in ND remains to be investigated.
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Conclusion

It is well recognized that the regulation of energy metabolism is highly compartmentalized in the brain and that the different brain cell types have distinctive metabolic profiles. However, the causality and functional consequences of the metabolic/mitochondrial changes that occur early in the course of many NDs is still debatable. However, they constitute an important readout of brain function/dysfunction and thus may be interesting brain biomarkers, especially when they occur at the pre-symptomatic stage of the disease, as in HD^{68, 117}. In some cases, they may also point to innovative, and safe, therapeutic targets that can be exploited at the earliest stages of the disease¹¹⁸. Research should focus on obtaining a better understanding the underlying mechanisms (focusing particularly on cellular aspects) and optimizing imaging/detection tools to more broadly assess energy metabolism *in vivo* in animal models and, ultimately, in patients.

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Titles and legends to figures

Figure 1. Schematic overview of some metabolic interactions between neurons, oligodendrocytes and astrocytes

Glucose circulating in the blood is taken up by astrocytes and oligodendrocytes (and also by neurons) and can be metabolized via glycolysis to produce lactate which can be then released and taken up by neurons, via specific monocarboxylate transporters located in astrocytes, synapses, myelin and axons. Lactate is subsequently oxidized in the tricarboxylic acid (TCA) cycle to produce ATP or can serve as a signaling molecule for neuronal plasticity. L-serine, synthesized in astrocytes from 3-phosphoglycerate, a glycolytic intermediate, can be shuttled to neurons via specific sodium-dependent and sodium-independent neutral amino acid transporters and converted to D-serine by the action of the serine racemase. D-serine is a co-agonist of the NMDA receptors and is involved in synaptic plasticity. The creatine/phosphocreatine (Cr/PCr) system is also highly compartmentalized in the brain. Creatine is essentially synthesized in glial cells (oligodendrocytes and astrocytes) that express AGAT and GAMT (especially oligodendrocytes) while neurons express high levels of both the Cl⁻-dependent creatine transporter and the mitochondrial (oxidative) creatine kinase (uMT-CK). Of note, the cytoplasmic (glycolytic) creatine kinase (B-CK) is mainly expressed in astrocytes. Considering the pivotal role of astrocytes (and oligodendrocytes) in brain homeostasis and the strong metabolic cooperation that exists between these glial cells and neurons, one need to better decipher how dysfunction of this metabolic dialogue may cause and/or contribute to neurodegenerative processes.

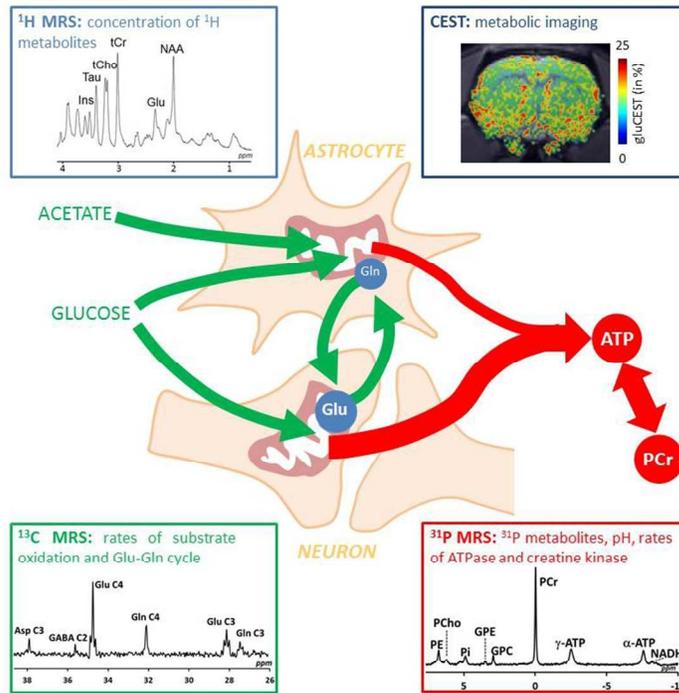
Figure 2. Use of NMR to probe energy metabolism

It is possible to investigate various aspects of brain metabolism non-invasively thanks to the versatility of *in vivo* nuclear magnetic resonance (NMR). ¹H magnetic resonance spectroscopy (MRS) yields the concentration of many metabolites in a given volume of interest. Chemical Exchange Saturation Transfer (CEST) imaging provides maps of the distribution of some metabolites (such as glutamate) that have hydrogen nuclei in fast exchange with water molecules. Beyond concentrations, it is also possible to quantify metabolic fluxes associated with mitochondrial function. ¹³C MRS, which relies on the infusion of ¹³C-labeled substrates and on the dynamic detection of ¹³C incorporation into metabolic by-products (mostly glutamate and glutamine) is used to quantify tricarboxylic acid (TCA) cycle in neurons and astrocytes, as well as glutamate-glutamine cycle. ³¹P MRS, combined with a technique called magnetization transfer, can be used to quantify the reaction rates of creatine kinase and ATP synthase.

Table 1. Advantages and limitations of the current non-invasive imaging modalities to probe energy metabolism defects in ND

Imaging modalities		Advantages	Limitations	Use in ND
MRS	^1H	Quantification of metabolites	Limited number of metabolites	AD, PD, HD
	^{31}P	Short acquisition time	Lack of standardization	HD, PD
		Direct measurement of energy metabolites (ATP) + synthesis	Low sensibility	
^{13}C	Cellular compartmentation	Cost	no	
	Long acquisition time			
CEST	Glucose	Imaging	Specificity	no
	Glutamate	Spatial resolution	Quantification	
fMRI	BOLD	Temporal and spatial resolution	Mix of vascular and oxidative metabolism	AD, PD, HD (activation and resting state)
PET	CMRGlu	Absolute quantification of glucose use	Low spatial resolution No indication about the fate of glucose	AD, PD, HD
	CMRO ₂	Absolute quantification of oxidative metabolism	Very low spatial resolution	AD, PD, HD

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