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The Journey From Metabolic Profiling to Biomarkers: The Potential of NMR Spectroscopy Based Metabolomics in Neurodegenerative Disease Research

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Abstract: Neurodegenerative diseases have become a "hot" topic in recent years. A major factor for this is that as life expectancy of the population in developed countries increases, so does the probability of developing neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD), to name the two most well-known. In many cases, however, neurological and mental diseases are poorly understood. In particular, there is a lack of specific biomarkers which would allow early unambiguous identification of a neurodegenerative disease, distinguishing between e.g. AD; PD; PD with dementia; and Dementia with Lewy bodies, or indicating therapeutic effects. Ultimately, this complicates the search for effective treatments. Thus, there is a high demand for preclinical work to elucidate underlying disease mechanisms and pave the way for disease management.

In terms of biomarker research, hope has been set on small molecules that participate in metabolism, since they provide a closer link between cellular mechanisms (with genetic as well as environmental inputs) and the disease phenotype. More specifically, it is expected that not one but a combination of several metabolites may serve as an indicator for disease onset and progression, given that neurodegenerative diseases, whilst often described as "idiopathic", are understood to arise from complex pathologies expressing themselves with a broad spectrum of phenotypes. Therefore, non-targeted metabolic profiling appears to offer great potential for biomarker discovery in this area.

One of the major technical platforms for non-targeted metabolic profiling is high resolution nuclear magnetic resonance (NMR) spectroscopy, a technique that is also available for the non-invasive application *in vivo*. Hence, in theory, biomarker discovery research using NMR spectroscopy based metabolomics provides a promising means for translation from *in vitro/ex vivo* research to eventual clinical use. This review will therefore discuss the potential for NMR spectroscopy based metabolomics to be applied to biomarker discovery in the field of neurodegenerative disease.

Keywords: Brain, metabolites, metabolomics, metabonomics, metabolic profiling, NMR spectroscopy, neurodegenerative diseases.

INTRODUCTION

Metabolic Profiling of Brain and its Diseases

Numerous publications in recent years demonstrate the successful application of metabolic profiling techniques to biofluids and tissues for the purposes of characterising metabolic perturbations associated with diseased states. These include cardiovascular conditions e.g. [1-4], disorders associated with metabolic syndrome [5-8] and cancer [9] to name but a few. In most cases, multifactorial metabolic changes were identified in those disorders suggestive of the human biocomplexity and indicating that more than just one singular marker is necessary for their comprehension.

The application of metabolic profiling techniques to brain diseases has consequently been proposed as a novel approach to shed light onto cellular mechanisms preceding, underlying and following complex neurological and mental conditions. Most of these disorders are incompletely understood, resulting in poor diagnostic accuracy and unsatisfactory disease management [10, 11]. Although often explained as "idiopathic" (of unknown origin), genetic predisposition as well as environmental inputs are believed to contribute to a number of neurodegenerative diseases and psychiatric illnesses expressing themselves in altered gene expressions and proteinaceous states [12, 13]. Given that the metabolic state of a biological system, at least in principle, incorporates both genetic (including transcriptional and proteinaceous) and environmental influences, metabolic profiling techniques, with their power to capture information relating to a great number of metabolites in a single analytical run, should be well placed to aid our understanding of the biochemical perturbations associated with these diseases. It is therefore hoped that this relatively novel approach aids the metabolic characterisation of central nervous system (CNS) diseases and hence, the search for valid biomarkers of brain disease onset, progression or therapeutic effects. Further, given the recent late-development stage failure of several AD drugs from pharmaceutical companies, Pfizer, Johnson & Johnson (J&J) and Elan, in Phase III trials, the potential value of a metabolomics approach within this field is clear [14].

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Investigations of metabolism in the CNS have proven to be complex, given the inaccessibility of the brain. The use of peripheral biofluids as diagnostic media may be questioned to some extent, because direct communication between the CNS and the periphery is hampered by the blood-brain barrier. Nevertheless, the use of blood plasma, serum and urine samples have been reported to determine altered metabolic profiles in schizophrenia [15], Parkinson's disease (PD) [16-18] and Alzheimer's disease (AD) [19].

Alternatively, cerebrospinal fluid (CSF) is in direct contact with the brain and its samples may therefore reflect the extracellular state of the CNS [20, 21]. Metabolic profiles of CSF have been established for multiple sclerosis [22] and degenerative dementias [23] including AD [24], PD [25] and Huntington's disease [26]. However, the collection of this central biofluid is not trivial and associated with some health risks. It is not collected routinely during clinical checks, therefore samples are not universally available. Access to samples from controls, healthy individuals is also problematic due to ethical issues associated with sample collection where pain and health risks are involved. Additionally, CSF collection is usually carried out *via* lumbar puncture and given its slow flow rate, may not necessarily provide an accurate representation of the metabolic processes in the brain [27]. This is reflected in the notion that lumbar and ventricular cerebrospinal fluids are not identical [11]. A further complicating factor is that the brain is a complex, multi-compartmental organ, and neurological diseases often affect only particular regions directly. However, metabolic profiles of the cerebrospinal fluid are not region-specific. Therefore, sampling tissues of specific brain areas are perhaps the most suitable matrix for investigating cerebral diseases in order to obtain localised metabolic information.

NMR Spectroscopy in Brain Diseases

The development of *in vivo* magnetic resonance spectroscopy (MRS) has enabled the non-invasive recording of the metabolic contents of the whole brain or targeted regions within it. However, it suffers from poor sensitivity and spectral resolution given relatively low magnetic field strengths and the semi-solid texture of the tissue, and can therefore only provide information about abundant metabolites (e.g.

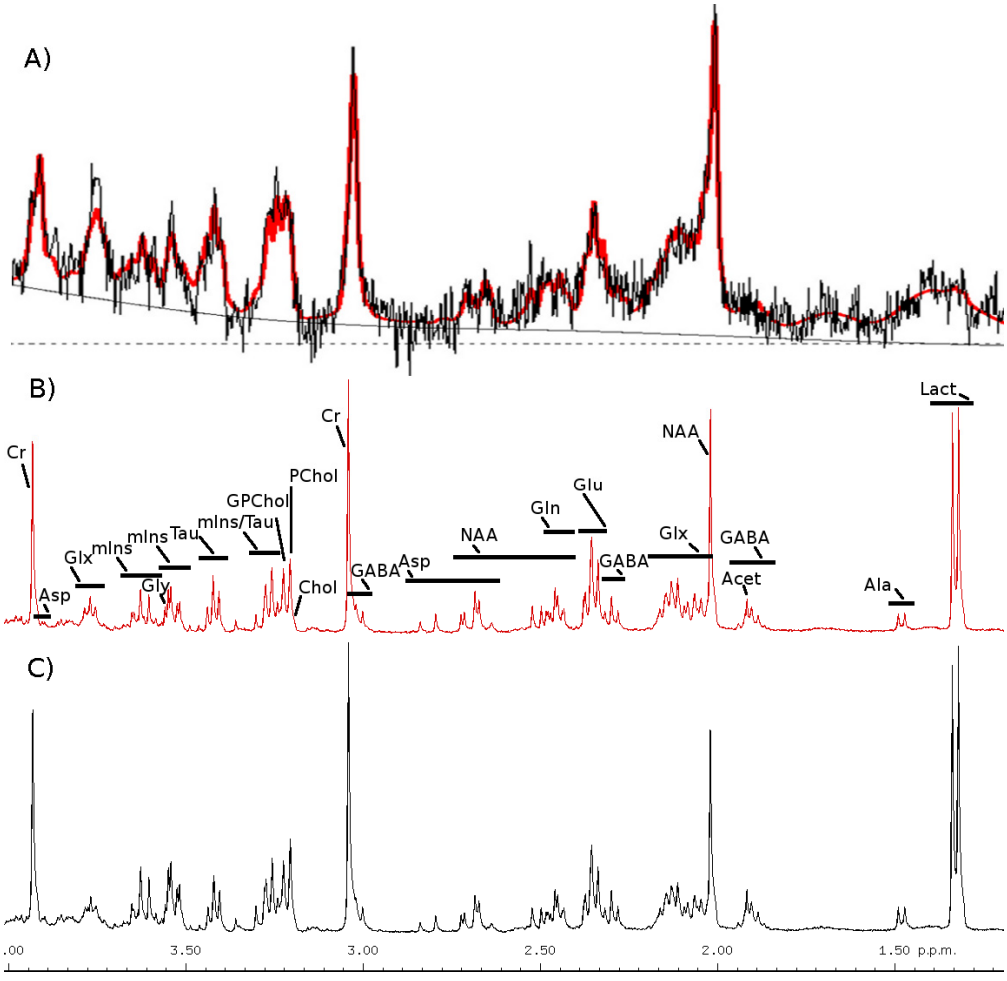


Fig. (1). *In vivo* A) and *ex vivo* B), C) spectra of rat brain extracts, where A) and B) show the frontal cortex, whilst C) shows the hippocampus. The *in vivo* spectrum was acquired at 7T using a wide bore magnet. The *ex vivo* spectrum was obtained from a methanol extract using a 5 mm probe at 9.7 T. KEY: Lact lactate, Ala alanine, GABA γ-aminobutyric acid, NAA N-acetylaspartic acid, Glx glutamate (Glu) and glutamine (Gln), Asp aspartate, Cr creatine, Chol choline, PChol phosphocholine GPChol glycerophosphocholine, Tau taurine, mIns μ-inositol, Gly glycine.

glutamate) or those whose chemical structures give rise to prominent NMR spectra, such as N-acetylaspartate (NAA). Despite these shortcomings, MRS has still proven to be useful in terms of both research and clinical application of brain disorders such as AD [28, 29], disorders related to PD [30], brain tumours and abscesses [31, 32], cerebral ischemia [33-35], schizophrenia [36] and anxiety [37].

The application of high resolution NMR spectroscopy to excised brain tissues or their extracts provides a picture of cerebral metabolism *ex vivo*. Of course, one disadvantage of *ex vivo* NMR spectroscopy of brain tissue is that collecting these samples is maximally invasive, hence, unethical in humans except where there is clinical necessity to remove tissue e.g. due to tumour growth. This clearly prohibits longitudinal investigations, which are only possible using biofluids or *in vivo* MRS. On the other hand, NMR spectroscopy is less expensive and more sensitive and is therefore capable of informing *in vivo* studies. The spectral difference between an *in vivo* (brain tissue of a living rat) and an *ex vivo* (rat brain extract) acquisition is illustrated in (Fig **1**).

Post-mortem investigations of brain tissues using *ex vivo* NMR spectroscopy have been applied to *e.g*. samples from demented patients [38-40] or schizophrenic subjects [41]. However, human post-mortem samples are representative of the late stages of progressive diseases and therefore information relating to the early stages of disease is inaccessible. A second issue is that post-mortem intervals (the time between death and storage of the tissue during which degradation processes occur and the metabolic profile of the tissue consequently changes) are difficult to control, although the introduction of "brain banks" may have improved post-mortem procedures [11].

In contrast to human samples, brain tissues can be excised from animal models of CNS diseases. These models can prove useful for investigation because they, (i) display particular characteristics of disease mechanisms and phenotypes, and (ii) their pathological signs may progress with time, which allows evaluation of different disease stages. They are, however, 'just' models of the human disease and will never cover the full spectrum of phenotypes and histopathologies that are observed in humans. Thus, translation of results from animal models to humans must be treated with caution.

To name a few studies that showed metabolic changes specific to brain tissue samples of animal models of cerebral diseases and conditions in contrast to control tissues: models of amyloidosis (relating to AD) [42, 43], Pick disease [44], PD [45], amyotrophic lateral sclerosis [46], Duchenne muscular dystrophy [47], Batten disease [48-50], Huntington's disease [51-53], spinocerebellar ataxia [54], brain tumours [55-57], traumatic brain injury [58] and chronic mild stress [59].

NMR SPECTROSCOPY-VISIBLE METABOLITES IN THE BRAIN AND THEIR FUNCTION

The metabolite type that can be investigated by means of NMR spectroscopy is dependent on the instrumental set-up and experiment type. However, assuming suitable tissue volume and length of spectral acquisition, approximately thirty metabolites can be identified in spectra obtained from hydrophilic cerebral tissue extracts. The functions of these metabolite are summarised in Table **1** together with their typical concentrations in 'normal' human brain tissue. Metabolite structures and spectral assignments with chemical shifts and coupling patterns are given in (Fig **2**) and Table **2**, respectively.

N-Acetylaspartate (NAA)

With normal concentrations ranging from 7.9 to 16.6 mmol/kg wet tissue, the acetylated amino acid NAA is one of the most abundant metabolites in the human brain, second to glutamate [60]. The metabolite concentration in rat cerebra is comparable at approximately 10 mmol/kg tissue [61]. The dipeptide neuromodulator N-acetylaspartylglutamate contributes to the *in vivo* MRS signal arising from the $–CH_3$ moiety of NAA at 2.02 ppm and overlaps with the glutamate resonances in the *ex vivo* spectra. N-acetylaspartylglutamate is present at around 10% of the NAA levels [62] and is thus not always unambiguously identifiable in NMR spectra.

Within species or single subjects, the concentration of NAA varies across different brain areas, and this metabolite is more abundant in grey than white matter [63]. In the rat, NAA undergoes developmental changes, increasing in the first three weeks of life to adult concentrations which gradually decrease with advancing age [62, 64, 65]. Similar trends have also been observed in humans [66, 67]. There appear to be concentration differences between genders in rats, with males displaying higher levels in the frontal and temporal cortex than females, implying hormone related variability in NAA metabolism [64], However, no gender differences have been found in humans [66]. Supporting the latter observation, metabolic profiles of mouse brain tissues that included NAA also did not appear to be influenced by gender [43, 45].

It is important to note that *in vivo* measurement of NAA concentration may deviate from *ex vivo* measurements because degradation processes reduce the abundance of this metabolite by fifty per cent within the first six hours *post-mortem* [68]. However, the sum of the *ex vivo* NAA concentration and that of one of its degradation products, acetate, appears to remain constant and is proposed to indicate the maximal NAA concentration *in vivo* [69].

In an energy-dependent fashion, it is suggested that NAA is synthesised in neuronal mitochondria where it acts as a precursor for the excitatory neurotransmitter and amino acid aspartate and acetate as well as N-acetylaspartylglutamate. As such, it provides a means for transporting aspartate and acetate from neurons to glia cells. NAA is also implicated in the formation of myelin lipids in glia cells through acetate [70].

Constituting 10% of all the anions in the brain, NAA makes up for the anion deficit in neurons [65] and acts as a neuronal osmolyte [70], protecting neurons from osmosis- and pH- related damage [63]; however, its neuronal volume and water homeostasis regulating role in the brain is rather subsidiary compared to one of the more dominant osmoregulators such as, taurine, μ-inositol, glutamate, glutamine and choline [70].

Table 1. A summary of brain metabolites - their typical concentration and function in mammalian brains. Normal human concentrations are given in mmol/kg of wet tissue and are reproduced from [60].

Metabolite	Normal Concentrations in Humans (mmol/kg of wet tissue)	Main Functions
Acetate	0.4 - 0.8	Fatty acid and steroid biosynthesis, precursor of neurotransmitter acetylcholine
N-Acetylaspartate (NAA)	7.9 - 16.6	Precursor of acetate, aspartate, N-acetylaspartylglutamate; ion balance and osmosis in neurons; neuronal marker
N-Acetylaspartyl-glutamate	0.6 - 2.7	Neuromodulator, group II metabotrophic glutamate receptors
Adenosine	3.0	Nucleoside, adenosine triphosphate (ATP) degradation product; energy metabolism; neuromodulator
Alanine	0.2 - 1.4	Amino acid: protein synthesis, amino acid/nitrogen transport across organs
γ-Aminobutyric acid	1.3 - 1.9	Inhibitory neurotransmitter
Creatine	5.1 - 10.6	Energy metabolism
Glutamate	6.0 - 12.5	Excitatory neurotransmitter, TCA cycle
Glutamine	3.0 - 5.8	TCA cycle, storage form of glutamate, hence regulating its activity; detoxification; amino acid/nitrogen transport across organs
Glycerol	< 0.1	Triglyceride core, phospholipid, hence membrane constituent
Glycerophosphocholine	1.0	Cell membrane constituent, osmoregulator
Glycine	0.1 - 1.0	Inhibitory neurotransmitter, antioxidant
Histidine	0.09	Precursor of neuromodulator histamine, amino acid: protein synthesis, chelating agent
μ-Inositol	3.8 - 8.1	(glial) osmoregulator; storage form of glucose; implicated in central secondary messenger system, hormonal stimulation, precursor of membrane constituents, glial marker
Scyllo-Inositol	0.0 - 0.6	Modulates protein folding
Lactate	0.4	End-product of anaerobic glycolysis; neuronal energy generation; macrophage activation
Phosphocholine	0.6	Cell membrane constituent
Phosphocreatine	3.2 - 5.5	Energy metabolism
Phenylalanine	< 0.1	Amino acid, catecholamine synthesis
Serine	0.4	L-serine; amino acid, glycine precursor D-serine: NMDA co-ligand (glycine binding site)
Succinate	0.4	TCA cycle
Taurine	0.9 = 1.5	Neuroprotective; blockage of glutamatergic NMDA receptors; osmoregulation; neuromodulator
Tyrosine	< 0.1	Amino acid; catecholamine synthesis
Valine	0.1	Amino acid: proteinsynthesis; propionic fatty acid precursor

Table 2. Resonance assignments with chemical shifts and spin-spin coupling patterns of metabolites commonly observed using *in vivo* and *ex vivo* spectroscopy of brain and brain extracts. * for proton identification see structures in Fig. (2). Spectroscopic data was acquired with standard compounds dissolved in D_2O at pH 7.2 using a Bruker Avance spectrometer (400 MHz) and then simulated using the Automated Consistency Analysis (ACA) software by PERCH Solutions Ltd. The spectral data of the metabolites marked with [a] were obtained from [60], those with [b] from the Human Metabolome Library (www.metabolibrary.ca/) and those with [c] from in-house databases. ATP was used as example for adenosine nucleotides. KEY: n(H) number of protons contributing to the signal; s singlet, d doublet, dd double doublet, triplet, qa quartet, qi quintet, m multiplet.

Metabolite	Chemical Moiety	Nucleus Identity*	n(H)	Signal Multiplicity	Chemical Shift [ppm]	*J*-Coupling [Hz]	Connectivity*
Acetate	CH_3	H^2	3	s	1.9250	--	--
N-Acetylaspartate (NAA)	CH_3	H^9	3	s	2.0223	3.722	H^4-H^{5A}
	CH_2	H^{5B}	1	dd	2.5062	10.134	H^4-H^{5B}
	CH_2	H^{5A}	1	dd	2.6991	0.208	H^4-H^9
	CH	H^4	1	dd	4.3969	-15.669	H^{5A}-H^{5B}
N-Acetyl-aspartyl-glutamate [a]	CH_2	H^{5A}	1	m	1.8810	4.412	H^2-H^{3A}
	CH_3	H^1	3	s	2.0420	9.515	H^2-H^{3B}
	CH_2	H^{5B}	1	m	2.0490	-15.910	H^{3A}-H^{3B}
	CH_2	H^{6B}	1	m	2.1800		
	CH_2	H^{6A}	1	m	2.1900		
	CH_2	H^{3B}	1	dd	2.5190		
	CH_2	H^{3A}	1	dd	2.7210		
	CH	H^2	1	dd	4.0607		
	CH	H^4	1	dd	4.1280		
Adenosine [a,b]	CH_2	H^{17A}	1	dd	3.8570	6.020	H^2-H^3
	CH_2	H^{17B}	1	dd	3.9370	5.030	H^3-H^4
	CH	H^5	1	q	4.3000	3.460	H^4-H^5
	CH	H^4	1	dd	4.4380	3.500	H^5-H^{17A}
	CH	H^3	1	s	4.8080	2.700	H^5-H^{17B}
	CH	H^2	1	d	6.0210	-11.800	H^{17A}-H^{17B}
	CH	H^7	1	s	8.1170		
	CH	H^{12}	1	s	8.2810		
ATP [a]	CH_2	H^{17B}	1	dd	4.2060	5.700	H^2-H^3
	CH_2	H^{17A}	1	dd	4.2950	5.300	H^3-H^4
	CH	H^5	1	q	4.3960	3.800	H^4-H^5
	CH	H^4	1	dd	4.6160	3.000	H^5-H^{17A}
	CH	H^3	1	s	4.7960	3.100	H^5-H^{17B}
	CH	H^2	1	d	6.1290	-11.800	H^{17A}-H^{17B}
	CH	H^7	1	s	8.2340	1.900	H^5-P
	CH	H^{12}	1	s	8.5220	6.500	H^{17A}-P
						4.900	H^{17B}-P

(Table 2) contd…..

Metabolite	Chemical Moiety	Nucleus Identity*	n(H)	Signal Multiplicity	Chemical Shift [ppm]	*J*-Coupling [Hz]	Connectivity*
Alanine	CH_3	H^4	3	d	1.7839	7.258	H^3-H^4
	CH	H^3	1	q	3.7653		
γ-Aminobutyrate	CH_2	H^3	2	qi	1.9077	-14.306	H^3-H^3
	CH_2	H^4	2	t	2.3025	8.087	H^4-H^3
	CH_2	H^5	2	t	3.0164	6.683	H^3-H^4
						6.040	H^5-H^3
						9.141	H^3-H^5
						-18.405	H^4-H^4
						-13.166	H^5-H^5
Aspartate	CH_2	H^{5A}	1	dd	2.6860	8.620	H^4-H^{5A}
	CH_2	H^{5B}	1	dd	2.8165	3.659	H^4-H^{5B}
	CH	H^4	1	dd	3.9033	-17.482	H^{5A}-H^{5B}
Choline	$(CH_3)_3$	H^{4-6}	9	s	3.2071	-14.449	H^3-H^{3A}
	CH_2	H^7	1	m	3.5248	6.193	H^3-H^7
	CH_2	H^{7B}	1	m	3.5248	3.255	H^3-H^{7B}
	CH_2	H^3	1	m	4.0627	0.354	H^3-N^1
	CH_2	H^{3A}	1	m	4.0627	3.061	H^{3A}-H^7
	N	$^{14}N^1$	--	--	-199.(9)	6.228	H^{3A}-H^{7B}
						0.432	H^{3A}-N^1
Creatine	CH_3	H^6	3	s	3.0396	-0.125	H^5-H^6
	CH_2	H^5	2	s	3.9336		
Formate [c]	CH	H	1	s	8.4600	--	--
Fumarate [c]	CH	H	2	s	6.5300	--	--
Glutamate	CH_2	H^{4B}	1	m	2.0635	4.670	H^{4A}-H^5
	CH_2	H^{4A}	1	m	2.1361	8.697	H^{4A}-H^{6A}
	CH_2	H^{6A}	1	m	2.3494	7.022	H^{4A}-H^{6B}
	CH_2	H^{6B}	1	m	2.3662	7.238	H^{4B}-H^5
	CH	H^5	1	t	3.7624	6.291	H^{4B}-H^{6A}
						8.582	H^{4B}-H^{6B}
						-16.302	H^{6A}-H^{6B}
Glutamine	CH_2	H^{4A}	1	m	2.1358	6.582	H^{4A}-H^5
	CH_2	H^{4B}	1	m	2.1554	6.134	H^{4A}-H^{6A}
	CH_2	H^{6A}	1	m	2.4444	9.448	H^{4A}-H^{6B}
	CH_2	H^{6B}	1	m	2.4714	5.790	H^{4B}-H^5
	CH	H^5	1	t	3.7972	9.361	H^{4B}-H^{6A}
						6.239	H^{4B}-H^{6B}
						-16.009	H^{6A}-H^{6B}

(Table 2) contd…..

Metabolite	Chemical Moiety	Nucleus Identity*	n(H)	Signal Multiplicity	Chemical Shift [ppm]	*J*-Coupling [Hz]	Connectivity*
Glycerol	CH_2	$H^{5/6A}$	2	dd/dd	3.5627	6.610	H^{4}-$H^{5/6A}$
	CH_2	$H^{5/6B}$	2	dd/dd	3.6532	4.357	H^{4}-$H^{5/6B}$
	CH	H^{4}	1	m	3.7835	-11.800	$H^{5/6A}$-$H^{5/6B}$
Glycerophospho-choline	$(CH_3)_3$	H^{8-10}	9	s	3.2335	-13.506	H^{7A}-H^{7B}
	CH_2	H^{11A}	1	m	3.3444	10.396	H^{7A}-H^{11A}
	CH_2	H^{14B}	1	m	3.3622	4.428	H^{7A}-H^{11B}
	CH_2	H^{7B}	1	m	3.6096	4.234	H^{7B}-H^{11A}
	CH_2	H^{14A}	1	m	3.6792	10.454	H^{7B}-H^{11B}
	CH	H^{12}	1	m	3.6809	-12.980	H^{11A}-H^{11B}
	CH_2	H^{7A}	1	m	3.6832	6.355	H^{12}-H^{13A}
	CH_2	H^{13B}	1	m	3.9101	5.654	H^{12}-H^{13B}
	CH_2	H^{11B}	1	m	3.9225	7.823	H^{12}-H^{14A}
	CH_2	H^{13A}	1	m	4.3327	2.964	H^{12}-H^{14B}
						-12.827	H^{13A}-H^{13B}
Glycine	CH_2	H^{3}	2	s	3.5625	--	--
Histidine [a]	CH_2	H^{8B}	1	dd	3.1866	7.959	H^{7}-H^{8A}
	CH_2	H^{8A}	1	dd	3.2644	4.812	H^{7}-H^{8B}
	CH	H^{7}	1	dd	3.9959	-15.513	H^{8A}-H^{8B}
	CH	H^{5}	1	m	7.1030	1.070	H^{3}-NH
	CH	H^{3}	1	d	7.9010	1.200	H^{5}-NH
						0.720	H^{5}-$H^{8A/B}$
Isoleucine	CH_3	H^{7}	3	t	0.9430	3.949	H^{3}-H^{4}
	CH_3	H^{6}	3	d	1.0152	4.782	H^{3}-H^{5A}
	CH_2	H^{5B}	1	m	1.2492	9.349	H^{3}-H^{5B}
	CH_2	H^{5A}	1	m	1.4589	7.021	H^{3}-H^{6}
	CH	H^{3}	1	m	1.9692	-13.496	H^{5A}-H^{5B}
	CH	H^{4}	1	d	3.6549	7.477	H^{5A}-H^{7}
						7.372	H^{5B}-H^{7}
μ-Inositol	CH	H^{8}	1	t	3.2814	9.466	$H^{7/10}$-H^{8}
	CH	H^{9}	1	dd	3.5377	10.019	$H^{7/10}$-$H^{9/12}$
	CH	H^{12}	1	dd	3.5377	2.937	$H^{9/12}$-H^{11}
	CH	H^{7}	1	t	3.6242		
	CH	H^{10}	1	t	3.6242		
	CH	H^{11}	1	t	4.0641		
Scyllo-Inositol	CH	H^{7-12}	6	s	3.3510	2.590	H^{7-12}- H^{7-12}
Lactate	CH_3	H^{4}	3	d	1.3313	6.933	H^{3}- H^{4}
	CH	H^{3}	1	q	4.1140		

(Table 2) contd…..

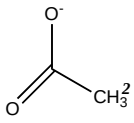
Metabolite	Chemical Moiety	Nucleus Identity*	n(H)	Signal Multiplicity	Chemical Shift [ppm]	*J*-Coupling [Hz]	Connectivity*
Leucine	CH_3	H^6	3	t	0.9591	8.994	H^3-H^{4A}
	CH_3	H^7	3	t	0.9708	9.315	H^3-H^{4B}
	CH_2	H^{4A}	1	m	1.6740	6.236	H^3-H^6
	CH	H^3	1	m	1.6977	6.216	H^3-H^7
	CH	H^5	1	t	3.7204	-16.323	H^{4A}-H^{4B}
	CH_2	H^{4B}	1	m	1.7279	8.997	H^{4A}-H^5
						4.701	H^{4B}-H^5
Nicotinamide[b]	CH	H^2	1	d	8.9400	2.170	H^2-H^6
	CH	H^4	1	m	8.2600	4.960	H^4-H^5
	CH	H^5	1	dd	7.5300	7.940	H^5-H^6
	CH	H^6	1	dd	8.8100		
Phosphocholine	CH_2	H^6	2	m	3.5743	-13.242	H^6-H^6
	$(CH_3)_3$	H^{7-9}	9	s	3.2264	2.543	H^{10}-H^6
	CH_2	H^{10}	2	m	4.1629	7.004	H^6-H^{10}
						-13.169	H^{10}-H^{10}
Phosphocreatine[a]	CH_3	H^6	3	s	3.0290	--	--
	CH_2	H^5	2	s	3.9300		
Phenylalanine[a]	CH_2	H^{8B}	1	dd	3.1132	5.209	H^7-H^{8A}
	CH_2	H^{8A}	1	dd	3.2827	8.013	H^7-H^{8B}
	CH	H^7	1	dd	3.9829	-14.573	H^{8A}-H^{8B}
	CH	H^2	1	m	7.3223	7.909	H^2-H^3
	CH	H^6	1	m	7.3223	1.592	H^2-H^4
	CH	H^4	1	m	7.3693	7.204	H^3-H^4
	CH	H^3	1	m	7.4201	0.493	H^2-H^5
	CH	H^5	1	m	7.4201	0.994	H^3-H^5
						7.534	H^4-H^5
						1.419	H^2-H^6
						0.462	H^3-H^6
						0.970	H^4-H^6
						7.350	H^5-H^6
Serine	CH	H^4	1	dd	3.8512	3.524	H^4-H^{6A}
	CH_2	H^{6B}	1	dd	3.9542	5.900	H^4-H^{6B}
	CH_2	H^{6A}	1	dd	3.9901	-12.293	H^{6A}-H^{6B}
Succinate	CH_2	H^1	4	s	2.4207	--	--

(Table 2) contd…..

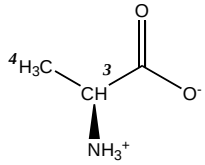
Metabolite	Chemical Moiety	Nucleus Identity*	n(H)	Signal Multiplicity	Chemical Shift [ppm]	*J*-Coupling [Hz]	Connectivity*
Taurine	CH_2	$H^{4A/B}$	2	t	3.2689	-19.931	H^{4A}-H^{4B}
	CH_2	$H^{5A/B}$	2	t	3.4312	8.113	H^{4B}-H^{5B}
						5.157	H^{4A}-H^{5B}
						-13.260	H^{5A}-H^{5B}
Triethanolamine	CH_2	H^{5-7}	6	t	3.1458	-13.493	H^{5-7}-H^{5-7}
	CH_2	H^{8-10}	6	t	3.8402	6.767	H^{8-10}-H^{5-7}
						3.766	H^{5-7}-H^{8-10}
						-12.689	H^{8-10}-H^{8-10}
Tyrosine [a]	CH_2	H^{8B}	1	dd	3.1132	5.147	H^{7}-H^{8A}
	CH_2	H^{8A}	1	dd	3.1908	7.877	H^{7}-H^{8B}
	CH	H^{7}	1	dd	3.9299	-14.726	H^{8A}-H^{8B}
	CH	H^{2}	1	m	6.9045	7.981	H^{2}-H^{3}
	CH	H^{6}	1	m	6.9045	0.311	H^{2}-H^{5}
	CH	H^{3}	1	m	7.0390	2.445	H^{3}-H^{5}
	CH	H^{5}	1	m	7.0390	2.538	H^{2}-H^{6}
						0.460	H^{3}-H^{6}
						8.649	H^{5}-H^{6}
Uracil [b]	CH_2	$H^{5A/B}$	2	d	5.7900	6.800	$H^{5A/B}$-$H^{6A/B}$
	CH_2	$H^{6A/B}$	2	d	7.5200	6.900	$H^{5A/B}$-$H^{6A/B}$
Valine	CH_3	H^{6}	3	d	0.9952	4.357	H^{3}-H^{4}
	CH_3	H^{5}	3	d	1.0464	7.039	H^{3}-H^{5}
	CH	H^{3}	1	m	2.2620	7.010	H^{3}-H^{6}
	CH	H^{4}	1	d	3.5930		

Whilst its physiological functions are still not completely understood, NAA has received clinical significance for its prominent signal in *in vivo* magnetic resonance (MR) spectra [71] that has been proposed as a marker for neuronal presence and viability for its predominant localisation in neurons [53, 60].

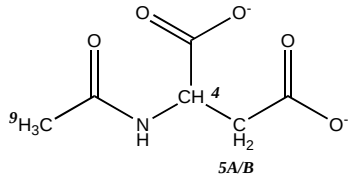
Through its putative role as a neuronal marker, NAA has been investigated for its significance in indicating and/or predicting the onset or course of neurodegenerative diseases. A reduction of NAA generally suggests neuronal dysfunction or cell loss [13], mitochondrial dysfunction e.g. via oxidative damage [53], or glial cell increase e.g. caused by neuroinflammation [42], whilst its increase may imply neuronal recovery [72]. Notably, NAA reductions have proven not to be specific to any particular neurodegenerative condition. For example, NAA was found to be decreased in the globus pallidus in panthothenate kinase associated neurodegeneration [73] and in the motor cortex of patients with amyotrophic lateral sclerosis [29]. There is also substantial evidence that compared to age-matched healthy controls, NAA levels are reduced *e.g.* in the medial temporal lobe in AD and patients with mild cognitive impairment (who have an increased probability of developing AD) which appeared to be weakly correlated with disease severity [28, 29 72]. Similarly, NAA has previously been shown to decrease in PD patients, albeit not severely enough for accurate diagnosis on this factor/parameter alone [74]. On the other hand, lower *in vivo* NAA-to-creatine ratios in the occipital lobes were proposed to be indicative of cognitive decline within PD patients [75], showing that the group of PD patients with dementia displayed significantly lower metabolite ratios than the group without dementia [76]. Although associated with the category of neurodegenerative dementing diseases, dementia with Lewy bodies expresses itself differently, by indicating normal NAA-to-creatine levels in the parasagittal parietal [77] and posterior cingulate cortex (where neuronal number and tissue volume remain unaltered) [78]. Decreases were found in the hippocampus [79] and the centrum semiovale white matter in early dementia with Lewy bodies patients which, however, did not correlate with disease severity or duration [77].



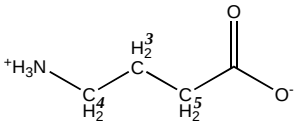
Acetate



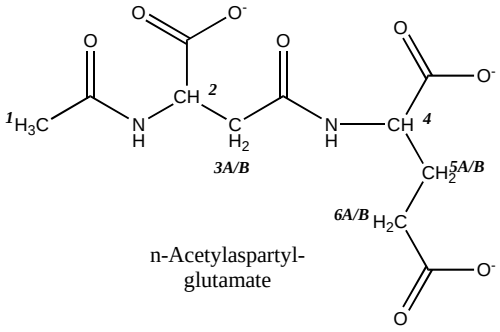
Alanine



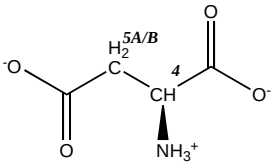
n-Acetylaspartate



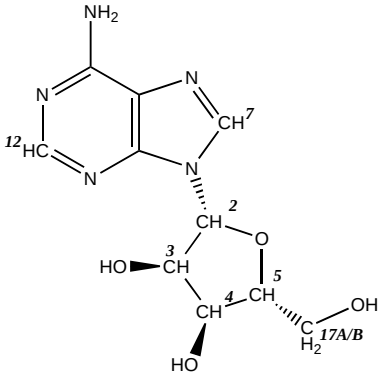
γ–Aminobutyrate



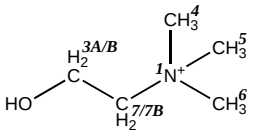
n-Acetylaspartyl-glutamate



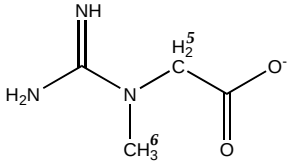
Aspartate



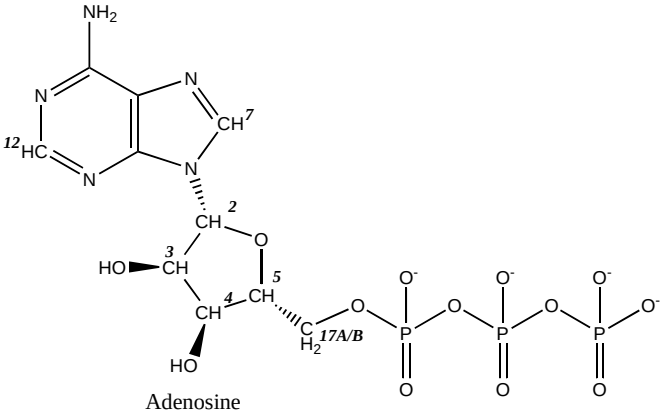
Adenosine



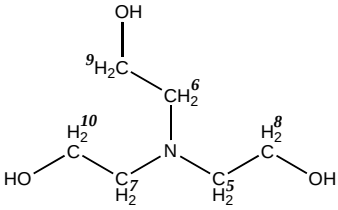
Choline



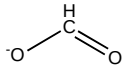
Creatine



Adenosine

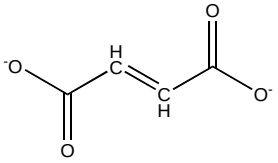


Triethanolamine

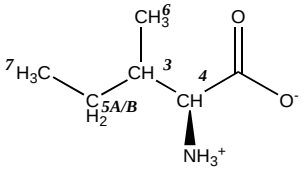


Formate

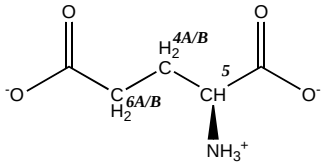
Fig. (2). Condt…..



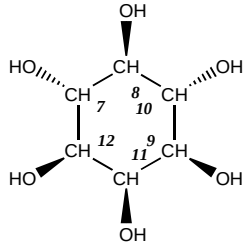
Fumarate



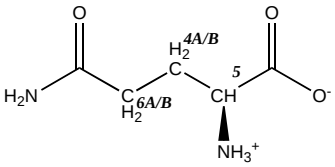
Isoleucine



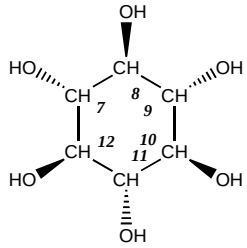
Glutamate



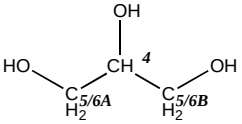
μ-Inositol



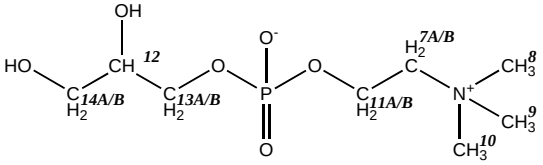
Glutamine



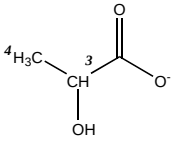
μ-Inositol



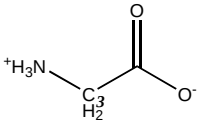
Glycerol



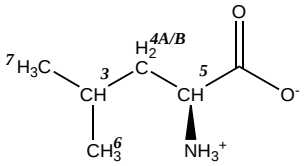
Glycerophosphocholine



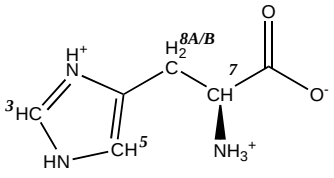
Lactate



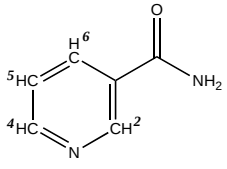
Glycine



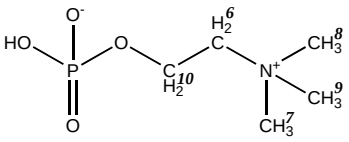
Leucine



Histidine



Nicotinamide



Phosphocholine

Fig. (2). Condt…..

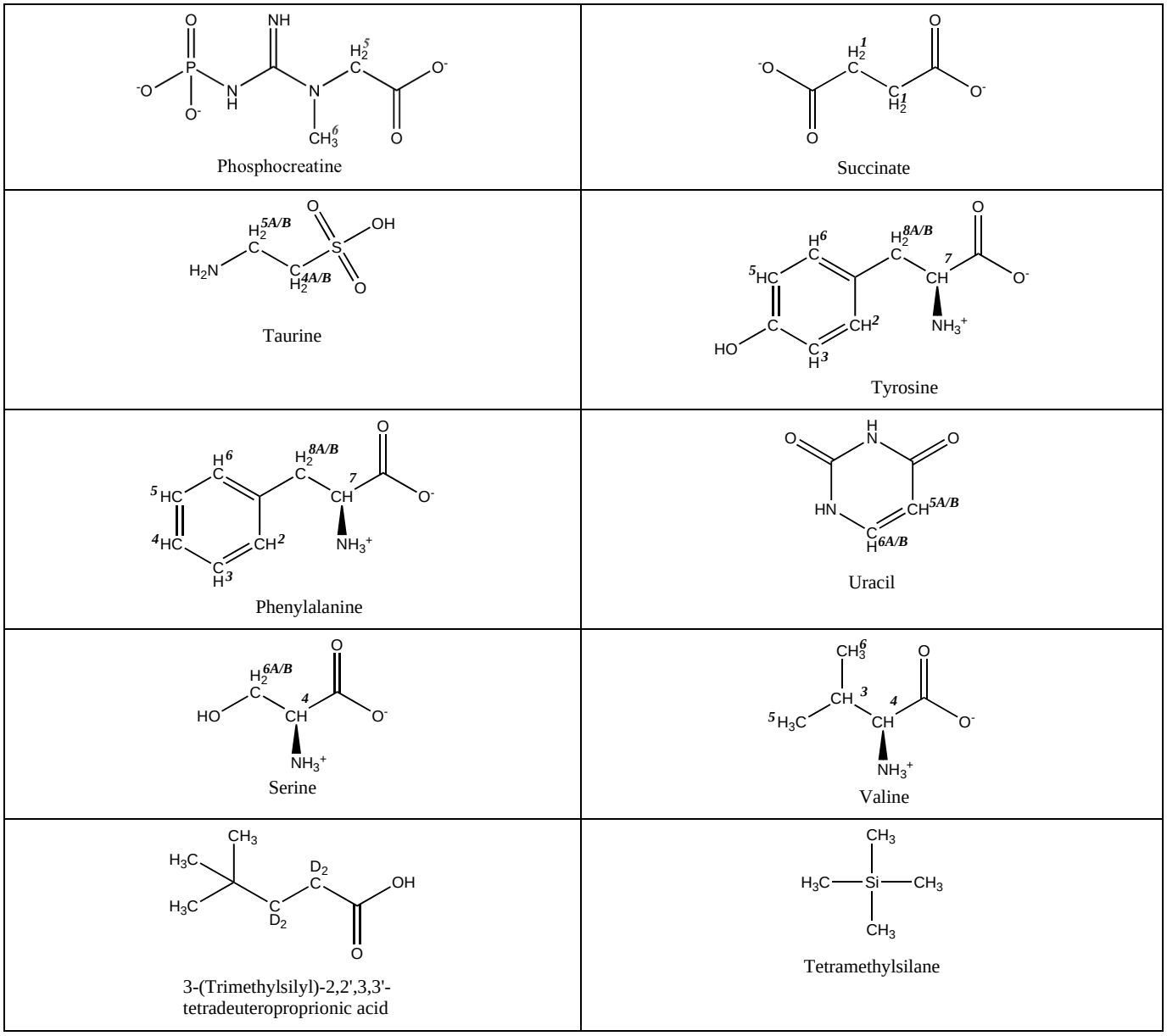


Fig. (2). Chemical structures of NMR spectroscopy visible metabolites relevant to metabolic profiling of the brain, including typical standards. ATP is used as example for adenosine nucleotides.

In animal models of neurodegenerative diseases, similar trends can be found for NAA: reduced levels were detected in the frontal cortex, remaining cortex and hippocampus of two single-transgenic mouse models of amyloidosis (a key feature of AD), whilst they remained unchanged in the midbrain [42, 43]. NAA has shown to be unaltered in several brain areas in a double-transgenic model of this very condition displaying more severe progression of this phenotype [80, 81]. Note, that these models do not display extensive neuronal loss as seen in human AD. Modelling PD, vesicular monoamine transporter (VMAT2)-deficient mice exhibited NAA reductions in the substantia nigra where a loss of dopaminergic neurons has been reported for PD [45]. In the same model, however, NAA concentrations were increased in the cerebellum, hippocampus, cortex and striatum, which may have resulted from increased aspartate levels and impaired amine metabolism [45]. In a transgenic mouse model of Huntington's disease, NAA reductions were detected before measurable neuronal loss occurred, but whilst neuronal inclusion bodies were present [51], demonstrating the potential of NAA as an early marker of neuronal degeneration.

Two curious conditions have been associated with extreme forms of altered NAA concentrations: (i) Canavan disease (or Hyperacetylaspartia), the only disorder described to date whose MRS signature includes significantly increased NAA signals, and (ii) Hypoacetylaspartia, an inborn disorder whose diagnosis is based on the total absence of NAA related signals in MR spectra of the human brain. Both diseases express themselves with severe disabilities. The latter condition has, so far, only been described in one boy, but would appear to demonstrate that the absence of NAA is not be to equated with non-survival of neurons or human life, it is, however, connected with drastic developmental delays and impairments [82, 83].

Creatine and Phosphocreatine

Phosphocreatine is the phosphorylated form of creatine, which is a nitrogenous organic acid. The enzyme creatine kinase maintains the equilibrium between these two compounds aiding the cellular energy homeostasis by phosphocreatine providing phosphate for generation of adenosine triphosphate (ATP) [29, 60].

Phosphocreatine and creatine are not usually distinguishable by *in vivo* MRS and are therefore reported as one entity. In humans, the concentrations of phosphocreatine and creatine have been estimated to be between 3.2 to 5.5 and 5.1 to 10.6 mmol/kg wet weight, respectively [60]. The levels in canine cerebra were also reported within this range, and the ratio between creatine and phosphocreatine was found to be 2.28 in parietal cerebral regions of adult beagle dogs [68].

After tissue excision, phosphocreatine degenerates rapidly [84] and is not usually observed in NMR spectra of cerebral tissue extracts, whereas creatine binds to macromolecules in the brain potentially reducing its extraction efficiency. Nonetheless, estimated concentrations of phosphocreatine and creatine combined *in vivo* have previously been reported to be comparable with those measured *ex vivo* [63, 68].

Creatine is synthesised through reaction of S-adenosylmethionine, ornithine and guanidinoacetate, the latter of which is the product of the amino acids glycine and arginine [85]. In contrast to NAA which is almost exclusively generated in the neurons of the CNS, the synthesis of creatine occurs in central as well as peripheral tissues such as the liver, kidney and pancreas [60, 63].

Within the brain, creatine occurs uniformly in neuronal and glial cell types and mitochondria, and is therefore generally considered suitable as an internal standard with "constant" concentration [71]. However, it has previously been shown that grey matter contains more phosphocreatine and creatine than white matter, and there is conflicting evidence as to whether age and gender affect creatine concentration [60, 63, 64].

In neurodegenerative diseases, creatine or phosphocreatine levels may not differ from values obtained from healthy controls, e.g. in AD, but do appear to be decreased in the motor neuron disease amyotrophic lateral sclerosis [29]. In pantothenate kinase-associated neurodegeneration, phosphocreatine and creatine levels in the globus pallidus are the same as control levels, but reduced in the basal ganglia when compared to normal tissue [73]. Over the course of amyotrophic lateral sclerosis and panthothenate-kinase associated neurodegeneration, the phosphocreatine and creatine concentrations decrease in specific brain areas [29, 73].

Glycerophosphocholine, Phosphocholine and Choline

In *in vivo* MRS the entities glycerophosphocholine, phosphocholine and choline are usually summarised as "(total) choline containing compounds" due to the inability to resolve the singlet arising from the $-N(CH_3)_3$ moiety of each of these molecules. In addition, carnitine [86] and glycerophosphorylethanolamine, phosphorylethanolamine and inositol resonances may also contribute to this signal [62]. *Ex vivo* NMR spectroscopy is preferentially carried out at field strengths of greater than 9.4 Tesla (T) and consequently, the choline and phosphocholine resonances are largely resolved from each other, with glycerophosphocholine resonance being close to that of phosphocholine. The quantification of glycerophosphocholine and phosphocholine is only possible *via* the use of deconvolution algorithms and not *via* direct integration of the peak area at magnetic field strengths between 14.1 T.

The concentration of choline-containing compounds ranges between 0.9 and 2.5 mmol/kg wet tissue in human cerebra; similar values were also reported for canine brain samples *in vivo* and *ex vivo* [60, 68]. *In vivo*, free choline contributes very little to the choline-containing compound signal [60]; *ex vivo*, on the other hand, choline alone gives rise to a prominent resonance at 3.02 ppm.

As an essential nutrient, choline is ingested through food intake [60]. In the body, it is implicated in the metabolism of one-carbon tetrahydrofolate derivatives, generating serine, glycine and purines, as well as methionine which is further converted to S-adenosylmethionine for methylation reactions [85]. Choline is further a precursor of the neurotransmitter acetylcholine; phosphocholine, which is a cell membrane constituent; and glycerophosphocholine, which is thought to be a cerebral osmolyte [60]. Because glycerophosphocholine and phosphocholine contribute to the majority of the choline-containing compound signal in *in vivo* MRS, this resonance is considered not to be related to acetylcholine abundance, but to reflect membrane compositions instead [60, 71].

In this sense, Klunk *et al*. [38] suggested an increase of choline-containing compounds as non-specific indication of neurodegeneration. However, it has been shown that their level remained constant in one study of PD and did not distinguish between those patients who additionally developed dementia and those who did not [76]; even though the number of cholinergic neurons was reduced in patients with PD plus dementia (or dementia with Lewy bodies) when compared with those without, or those diagnosed with AD [87]. In a mouse model of PD, choline-containing compounds were reduced in the substantia nigra [45] but, choline-to-creatine ratios have previously been described as normal in AD [29]. However, another study demonstrated, using *ex vivo* NMR spectroscopy, that different cholinergic constituents were changed (glycerophosphocholine and choline decreased, phosphocholine increased) whilst the joint choline-containing signal detectable via *in vivo* spectroscopy remained unaltered in AD [40]. In a separate study, glycerophosphocholine and phosphocholine levels were both reported to be increased in AD, especially at older age [38].

μ-Inositol and Scyllo-Inositol

An inositol is a cyclic sugar alcohol, a cyclohexanehexanol with a hydroxyl group bound to every carbon atom of the hexagonal ring. μ-Inositol and scyllo-inositol are epimers to each other with one hydroxyl group being orientated differently at one carbon centre. With a concentration range of 3.8 to 8.1 mmol/kg brain tissue, μ-inositol is far more abundant in the human brain than scyllo-inositol, which is present at typical concentrations of *ca*. 0.3-0.6

mmol/kg [60]. Because all six protons resonate at the same frequency, scyllo-inositol may be visible in the NMR spectra of brain extracts [71]. However, the scyllo-inositol singlet at around 3.35 ppm can be difficult to identify by purely spectroscopic methods and can easily be mistaken for methanol, which gives rise to a singlet at *ca.* 3.37 ppm in NMR spectra. Methanol is commonly used in combination with chloroform and water to extract tissue samples. μ-Inositol on the other hand gives rise to prominent complex signals in high resolution spectra of brain extracts, which are easier to identify. In *in vivo* spectra, μ-inositol peaks may be overlapped with glycine, glycerol, taurine and phosphorylated forms of inositols [60].

To date, the biological functions of μ-inositol and other inositols have not been fully elucidated. They are components of glycerophospholipids which are the most abundant lipids within membrane structures [85]. As a storage form and a degradation product of inositol-1,4,5-triphosphate, μ-inositol is also involved in this signal transduction system where inositol-1,4,5-triphosphate triggers the release of calcium ions from the endoplasmic reticulum and mitochondria [85, 88, 89]. One of μ-inositol's degradation products is glucuronic acid which may aid detoxification of the brain after drug administration [89]. It also takes on some function as a storage form of glucose and in cellular growth [45, 60, 89]. Given its predominance in glial cells, where it acts as osmoregulator *via* its transport across cell membranes, it is regarded as glial cell marker, with an increase indicating gliosis or inflammation; although high concentrations of μ-inositol have also been found in neuronal cell lines [76, 90].

Through its osmoregulatory role and implication on cell membrane structure, μ-inositol levels respond to brain injury impacts [60]. In PD with or without dementia, μ-inositol does not appear to be altered [76], although consistent reductions were found in the substantia nigra of the VMAT2 mouse model of PD [45]. Similarly, no changes have been reported for dementia with Lewy bodies [77, 78]. By contrast, changes have been found in AD [60, 90], where elevated μ-inositol levels were detected in the whole brain [91] and more specifically, in the posterior cingulate [92]. In a post-mortem study of cortices of human AD and non-AD demented patients including non-demented controls, μ-inositol levels in the AD group were not only higher than in controls but also higher than in non-AD demented patients [38]. Whether the μ-inositol is correlated with the number of senile amyloid plaques (a histological hallmark feature of AD) appears unclear [38, 93]. In most studies of double transgenic (APP and PS1) mouse models of amyloidosis, μ-inositol was found to be increased [81, 94-96].

Taurine

Taurine is a 2-aminoethanesulfonic acid that is produced from the amino acid cysteine, most likely via the compound hypotaurine [97]. There is some disagreement as to which cell types host taurine biosynthesis, but it appears that the *in vivo* generation of taurine is regulated via the cooperation of both neuronal and astrocytic glial cells [97]. Taurine levels can also be influenced by its consumption in certain foods [60].

Being abundant throughout the mammalian body, the dominant roles ascribed to taurine in the CNS are osmoregulation and neuroprotection and because it competes with glycine and γ-aminobutyric acid receptor binding sites, it has also been described as an inhibitory neuromodulator [98]. The neuroprotective effects of taurine are probably exerted via the reduction of cellular ion Ca^{2+} influx, which may be driven by glutamatergic neurotransmission or other mechanisms and also by scavenging free radicals in states of oxidative stress [98]. Changes in central taurine concentrations have been associated with an increased vulnerability to the effects of osmotic stress [97].

In *in vivo* MRS, taurine is less clearly identifiable in humans than in rodents given its reduced relative concentration of approximately 1.73 to 12.5 mg/kg in humans vs. 20.4 to 75.5 mg/kg in mice [41, 47, 51, 57] and therefore, few publications mention MRS based measurement of taurine in brain disease. It has been suggested that increased taurine levels may be indicative of excessive cellular proliferation in tumours [99] and may be useful for the discrimination of malignant and benign astrocytomas [57]. Taurine levels appeared to be unaffected in one and increased in another study investigating post-mortem cerebral tissues of schizophrenic patients [41, 100] and unaltered after cerebral ischaemia in rats [101]. Further, taurine was decreased in several brain regions of a transgenic mouse model of PD and in a single transgenic murine model of AD [43, 45]; however, taurine has also been found to be increased in another single transgenic mouse model of amyloidosis [42]. Taurine levels are unaffected in tissues collected from patients with AD post-mortem [38, 39].

Glutamate and Glutamine

Glutamate and glutamine are directly related amino acids, where the side-chain carboxyl group in the glutamate molecule is replaced by an amide moiety in glutamine. With a concentration range of 6.0-12.5 mmol/kg, glutamate is more abundant than glutamine (3.0-5.8 mmol/kg) in the human brain; comparable with values reported for mouse cerebra [51, 89, 102].

In the CNS, glutamate is an interesting molecule to investigate for its dual role: (i) it is active as an excitatory neurotransmitter; (ii) it takes part in metabolic processes, together with glutamine [102]. Metabolic glutamate is mainly found in neurons, but also in glia, whilst the neurotransmitter, glutamate is only found in vesicles within glutamatergic neurons [86, 102]. After the neurotransmitter glutamate is released into the synaptic cleft to perform its excitatory action, it is taken up into glial cells (astrocytes), where it is converted to its storage form glutamine by the glutamine synthetase under the expense of ATP [89, 102]. Glutamate can become available as a neurotransmitter again after glutamine is transported into neuronal cells, in whose mitochondria it is metabolised to glutamate by the glutaminase enzyme [89]. The glutamate transport into its neurotransmitter vesicles is ATP dependent and unidirectional [39, 102]. Besides its role as a storage form and provider of glutamate, glutamine may also be implicated in oxidative metabolism [51].

Using *in vivo* and *ex vivo* NMR spectroscopy, it is difficult to identify the resonances relevant to glutamatergic neurotransmission. Firstly, glutamate and glutamine resonances are often indistinguishable using *in vivo* MRS, especially at field strengths less than 3 T. In this case, they are not considered as separate entities, but as a combined signal "*Glx*". Secondly, there is some doubt that glutamate signals themselves are representative of the neurotransmitter pool, given its packed location within vesicles [71, 103]. Rather, glutamine levels may indicate the state of the neurotransmitter glutamate, as 90% of the glutamine pool is generated by the neurotransmitter glutamate taken up into astrocytes [102].

It is reported that the glutamate/glutamine ratio may be reduced in AD cerebra [80]. In concordance, glutamine appeared to be increased in the cerebrospinal fluid of demented patients [23]. Glutamate was found to be decreased within all brain regions of three mouse models of amyloidosis [42, 43, 95, 104, 105]. However, in post-mortem brain tissue from patients with AD, glutamate was found to be increased [38]. Glutamine levels remained unchanged in one mouse model of amyloidosis [42], but decreased in another when compared to wild-type animals [45].

Post-mortem investigations of PD brains showed no difference of glutamine or glutamate levels compared to control brains, and they also did not discriminate between patients with or without dementia [106]. In living PD patients, increased *Glx* signals were observed [73]; but levels were found to be decreased in the posterior cingulate gyrus of patients with PD with dementia [92]. No consistent alterations of *Glx* levels were found in patients with dementia with Lewy bodies [72, 77]. In the VMAT2 model of PD, glutamate was increased, alongside other amino acids, whilst glutamine did not show any consistent changes [43].

It has previously been demonstrated that brain tissue from schizophrenic and control subjects could not be discriminated on the basis of glutamine and glutamate levels [41]. In different brain areas of a mouse model of amyotrophic lateral sclerosis, decreases of both glutamine and glutamate were found where the former was observed before disease onset [46]. In the cortex of a mouse model of Batten disease, glutamate levels were increased whilst glutamine concentration was decreased [49]. Changes the other way round were found in a sheep model of the same disease [50]. In mouse models of Huntington's disease, glutamate levels were decreased whilst glutamine levels were markedly increased [51, 52]. No changes in glutamine or glutamate were found in a mouse model of Duchenne muscular dystrophy [47]. In a murine model of prion disease, glutamate was found to be decreased whereas glutamine levels increased [107]. The latter was also observed in a transgenic mouse model of spinocerebellar ataxia 3 [54].

FUTURE PERSPECTIVES

The use of metabolic profiling techniques as an aid for biomarker research in neurological and mental health diseases has been promoted for a number of years [2, 108-110]. The main argument in its favour is the ability to screen multiple variables in a sample in a single analytical step. Early NMR spectroscopy and mass spectrometry investigations of post-mortem human and animal brains have characterised metabolic disturbances in a number of brain conditions (or models thereof) or shown potential disease risk factors. However, results have yet to find their way into regular clinical practice. One reason may lie in the complexity of the data analysis, which may be impractical in a clinical setting. Databases such as the Human Metabolome Database [111, 112] or MetaboLights [113] may go some way towards helping interpretation of individual compounds from mass and NMR spectra of biological samples. Another promising tool is a web-accessible decision support system, like eTumour [114], that aids brain tumour classification on the basis of *in vivo* MRS and MR imaging (MRI) data.

The Use of Animal Models of Brain Diseases

Pre-clinical research of animal models of brain diseases will continue to support the search for biomarkers in human diseases, but care must be taken in the interpretation of the data, considering that the animals will always only model certain aspects or mechanisms of the disorder, but never the full spectrum. There is a demand for models that mimic human conditions more closely, especially in AD, where amyloidosis models fail to reproduce the severe neuronal loss as seen in the human disease.

The application of metabolic profiling to animal models of brain disease is in its infancy and many published studies require validation by additional studies carried out on the same animal model, different models of the same disease or on the respective human condition. Future research in the field should also focus on exploring longitudinal changes in metabolism as pathologies progress and investigate how these patterns can be manipulated to ultimately find effective treatment regimes.

The Use of Peripheral Biofluids

The alternative to investigating cerebral metabolism directly is the use of human (or animal) peripheral biofluids e.g. blood plasma or serum. The use of these sample types is practical given their easy access and their relatively cheap analysis using *ex vivo* methods.

For their use in the analysis of brain diseases, the relationship between central and peripheral metabolism remains to be clarified in health and disease. For example, plasma and prefrontal cortical glutamate do not appear to be correlated with each other in healthy controls [115], posing a problem if one wanted to investigate glutamatergic metabolism or neurotransmission in the brain by analysing peripheral glutamate or glutamine concentrations. Future studies in this field will have to include a greater number of metabolites to provide a more comprehensive picture. *Ex vivo* NMR spectroscopy based metabolic profiling of brain tissue, cerebrospinal fluid and blood plasma may be a suitable choice of method to detect metabolite correlations, especially in combination with correlation matrices such as statistical total correlation spectroscopy (STOCSY) [116]. Also, combining *ex vivo* NMR spectroscopy of e.g. blood plasma with *in vivo* MRS or (structural or functional) MRI of the living brain may show a relationship between peripheral metabolism and cerebral parameters.

To date, few metabolic profiling studies have demonstrated that the sanguineous metabolic profiles are altered in neurodegenerative disorders, such as PD [17] and AD [117, 118]. These studies are primary steps towards the development of practical biomarkers for neurodegenerative diseases. The approaches by Tukiainen *et al.* [117] and Greenburg *et al.* [118] are particularly interesting because they focus on the lipid profile; a molecule class that is often overlooked by small-biochemical profiling, but highly relevant for brain diseases in which altered lipid metabolism is suspected, such as the case with AD [119]. Clearly more work is needed to verify their results using larger sample sizes. In addition, more diseases, or subgroups of diseases, have to be included in one study to validate the specificity of established markers for one particular condition. Once these points are satisfied, longitudinal studies will be required to clarify the behaviour of those markers in relation to disease progression, or under the influence of therapeutic interventions. Metabolic profiling of biofluids has already shown to be of great value to validate treatment effects, including the evaluation of desired drug efficacy and unwanted side-effects in pharma- and toxicological studies [120-122].

Information Provided by Metabolic Profiling Platforms

Metabolic profiling provides "snap-shots" of the metabolic state and can indicate the end-result pathological processes, but does not directly show which mechanism is malfunctioning. Hence, more studies are required which offer explanations for metabolic alterations e.g. by combining metabolic profiling techniques with platforms investigating the same systems on a "higher" level (proteins/enzymes or genes). It is hoped that correlation studies of metabolic aberrations and single nucleotide polymorphisms help elucidating the relationship between the genome and the metabolome. Another example would be "activity based metabolomic profiling" studies which have demonstrated their capacity to analyse the effect of specific enzymatic reactions on the metabolism of cellular extracts [123].

The use of different nuclei in NMR spectroscopy will broaden the spectrum of detectable metabolites. For example, ^{31}P NMR spectroscopy focuses on phosphorylated metabolites that participate in energy homeostasis of a cell. Study of the ^{13}C nucleus may be more interesting in terms of disease mechanism: in combination with dynamic nuclear polarisation (DNP), ^{13}C tracers can be used to visualise metabolic fluxes in real-time *in vivo*, as previously applied to myocardial metabolism by Schroeder *et al.* [124].

CONCLUSIONS

In conclusion, given the complexity and tightly controlled homeostasis of cerebral metabolism and the sensitivity limitations of *in vivo* MRS, it appears to be a difficult task for this technique to find a (single) diagnostic marker of neurodegenerative diseases, particularly at the pre-symptomatic stages. More realistic may be its application to finding a progression marker or a metabolic indicator of therapeutic action. The use of *ex vivo* NMR spectroscopy or mass spectrometry based metabolic profiling studies will, however, continue to aid the comprehension of pathological mechanisms in and the search for biomarkers of human brain diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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