HAL
open science

Expanded neurochemical profile in the early stage of Huntington disease using proton magnetic resonance spectroscopy

Isaac Mawusi Adanyeguh, Marie-Lorraine Monin, Daisy Rinaldi, Leorah Freeman, Alexandra Durr, Stéphane Lehéricy, Pierre-Gilles Henry, Fanny Mochel

To cite this version:

Isaac Mawusi Adanyeguh, Marie-Lorraine Monin, Daisy Rinaldi, Leorah Freeman, Alexandra Durr, et al.. Expanded neurochemical profile in the early stage of Huntington disease using proton magnetic resonance spectroscopy. NMR in Biomedicine, In press, 10.1002/nbm.3880 . hal-01688609

HAL Id: hal-01688609

https://hal.sorbonne-universite.fr/hal-01688609v1

Submitted on 19 Jan 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Expanded neurochemical profile in the early stage of Huntington disease using proton magnetic resonance spectroscopy

Isaac M. Adanyeguh[1], Marie-Lorraine Monin[1,2], Daisy Rinaldi[1], Léorah Freeman[3], Alexandra Durr[1,2], Stéphane Lehéricy[1,4], Pierre-Gilles Henry[5 *], Fanny Mochel[1,2,6 *]

[1] Inserm U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France

[2] AP-HP, Pitié-Salpêtrière University Hospital, Department of Genetics, Paris, France

[3] Department of Neurology, McGovern Medical School at UTHealth, Houston, TX, Unites States

[4] Center for NeuroImaging Research (CENIR), Institut du Cerveau et de la Moelle épinière, Paris, France

[5] Center for Magnetic Resonance Research (CMRR), University of Minnesota, Minneapolis, MN, United States

[6] University Pierre and Marie Curie, Neurometabolic Research Group, Paris, France

* These authors contributed equally to this work.

Correspondence to: Dr Fanny Mochel, Institut du Cerveau et de la Moelle épinière, Aile 4A, Hôpital Pitié-Salpétrière, 75013 Paris, France; Tel: +33 (0) 1 57 27 46 82; Fax: +33 (0) 1 57 27 47 95; email: fanny.mochel@upmc.fr

Word count: Abstract (243), main text (3287).

Grant support: This study was funded by NIH grants (P41EB015894 and P30NS076408) and the Ecole des Neurosciences de Paris. The study also received funding from Ipsen (NCT01696708) and the program "Investissements d'avenir" ANR-10-IAIHU-06 and ANR-11-INBS-0006.

Keywords: Huntington disease, movement disorders, ^{1}H MRS, semi-LASER, neurometabolite, neurochemical profile.

List of abbreviations:

AFP: Adiabatic full passage

HD: Huntington disease

CAG: Cytosine-adenine-guanine trinucleotide

UHDRS: Unified Huntington Disease Rating Scale

GM: Gray matter

WM: White matter

CSF: Cerebrospinal fluid

OVS: Outer volume suppression

VAPOR: Variable power and optimized relaxation delays

VOI: Volume of interest

RF: Radiofrequency

ABSTRACT

Purpose: The striatum is a well-known region affected in Huntington disease (HD). However, other regions including the visual cortex are implicated. We previously identified an abnormal energy response in the visual cortex of patients at an early stage of HD using ^{31}P magnetic resonance spectroscopy (MRS). We therefore sought to further characterize these metabolic alterations with ^{1}H MRS using a well-validated semi-LASER sequence that allows measuring an expanded number of neurometabolites.

Materials and methods: Ten early affected patients (UHDRS, total motor score = 13.6 ± 10.8) and ten healthy volunteers of similar age and BMI were recruited for the study. We performed ^{1}H MRS in the striatum – the region that is primary affected in HD – and the visual cortex.

Results: The protocol allowed a reliable quantification of 10 metabolites in the visual cortex and 8 in the striatum, compared to 3-5 metabolites in prior ^{1}H MRS studies performed in HD. We identified higher total creatine ($p < 0.05$) in the visual cortex and lower glutamate ($p < 0.001$) and total creatine ($p < 0.05$) in the striatum of HD patients compared to controls. Less abundant neurometabolites (glutamine, GABA, glutathione, aspartate) showed similar concentrations in both groups.

Conclusion: The protocol allowed measurement of several additional metabolites compared to standard vendor protocol. Our study pointed to early changes in metabolites involved in energy metabolism in the visual cortex and striatum of HD patients. Decreased striatal glutamate could reflect early neuronal dysfunction or impaired glutamatergic neurotransmission.

INTRODUCTION

Huntington disease (HD) is a polyglutamine disorder caused by expansion in the glutamine-encoding cysteine-adenine-guanine (CAG) repeats[1] and striatal atrophy is a prominent characteristic of the disease.[2] Other brain regions including the thalamus, cerebral cortex, cerebellum[3,4] and visual cortex[5] are also involved in the pathological process as the disease progresses. Positron emission tomography studies have shown metabolic alterations in the striatum[6] whilst ^{31}P MRS studies have revealed abnormal energy response in the visual cortex of HD patients at an early stage of the disease.[7,8] With the availability of genetic testing for HD, there exists a therapeutic window that can be taken advantage of before symptoms onset. However, biomarkers are critically needed to evaluate presymptomatic individuals and make use of this therapeutic window[5] since the Unified Huntington Disease Rating Scale (UHDRS),[10] the most commonly used measure to assess disease severity, is unable to evaluate pathological processes prior to motor symptoms onset. In the search for biomarkers, ^{1}H MRS has been used to study neurometabolism in HD at different field strengths.[11-15] Metabolic alterations start indeed in the very early stages of the disease,[16] and represent important targets for neuroprotective therapies.[8]

Previous studies with ^{1}H MRS in HD were carried out at field strengths between 0.5 T and 7 T (Table 1), with several studies performed on 3 T systems that are becoming common in hospitals.[12,13,15,17] In general, these studies have focused on the most prominent metabolites in the ^{1}H spectrum – total NAA (tNAA), total creatine (tCr), total choline (tCho), and to a lesser extent *myo*-inositol (*myo*-Ins) and glutamate (Glu) – but not on less obvious metabolites such as gamma amino-butyric acid (GABA), glutamine (Gln), or glutathione (GSH). Some studies reported metabolite ratios only, which further complicate data interpretation.[12,13] Moreover, most of these studies

focused mainly on the striatum, caudate or putamen, and only a few on the occipital cortex (Table 1) even though abnormal metabolic alterations are also present in this region.[6-8] Furthermore, these studies used STEAM and PRESS sequences with long or short T_E without prior test of robustness or reproducibility. The present study thus sought to measure an expanded neurochemical profile in both the visual cortex and the striatum of early affected HD patients at 3 T, using a previously optimised and validated semi-LASER sequence with water as an internal concentration reference and correction of concentrations for CSF content.

MATERIAL AND METHODS

The study was designed to test whether we can reliably report on an expanded range of neurochemicals in HD using a validated sequence with minimal J-modulation, small chemical-shift displacement error, higher SNR and excellent outer volume suppression performance compared to common vendor-provided sequences.

Subjects

The local ethics committee approved this study and all subjects signed a written informed consent after the nature of the procedure had been fully explained before participating in the study. Motor dysfunction was evaluated with the total motor score (TMS) of the Unified Huntington Disease Rating Scale (UHDRS) with a maximal worth score of 124. We recruited ten patients at the early stage of HD without medication as well as ten healthy volunteers with similar general characteristics – sex, age and BMI (Table 2).

MR Protocol

MRS data were acquired on 3 T whole-body Siemens Magnetom Trio scanner (Siemens Medical Solutions, Erlangen, Germany). We used a modified semi-LASER ^{1}H MRS protocol[18] that has been previously tested in healthy controls for robustness and reproducibility in a bi-centric study and demonstrated highly reproducible data.[19] Using a 32-channel receive head coil, 3D T_1-weighted volumetric images (T_R = 2530 ms, T_E = 3.65 ms, 1 mm isotropic, FOV = 256 x 240 mm^2, matrix size = 256 x 240) were acquired for spatial normalization and localization of brain volumes and for volumetric analysis of brain regions of interest. Shimming and spectra acquisition were performed on a 25 x 25 x 25 mm^3 volume-of-interest (VOI) in the visual cortex and a 34 x 19 x 23 mm^3 VOI in the striatum (Figure 1). Automatic B_0 shimming in the voxels was performed with FAST(EST)MAP[20] resulting in highly resolved spectral lines. The calibration and acquisition steps were performed as described in prior studies.[18,19,21] The RF power for the asymmetric slice-selective 90° pulse (duration = 2 ms) of the semi-LASER sequence (T_R = 5000 ms, T_E = 28 ms, averages = 64, vector size = 2048, acquisition time = 6 min) was calibrated by determining the power that produced the maximum signal. The power for the 180° hyperbolic secant adiabatic full passage (AFP) pulses (duration = 4 ms) was set automatically based on the 90° pulse. For better suppression of unwanted coherences with shorter spoiler gradients, AFP pulses were interleaved. Water suppression pulses with the variable pulse power and optimized relaxation delays (VAPOR) were adjusted by following signal intensities to determine the power with maximum water suppression. The VAPOR pulses were interleaved with outer volume suppression (OVS) pulses to reduce contamination from other brain regions outside the VOI in the visual cortex (thickness: anterior = 200 mm, posterior = 40 mm) and the striatum (thickness:

anterior = 80 mm, posterior = 80 mm). A 7 mm margin was left between the VOI and the OVS slices in order to avoid signal loss in the VOI due to the transition band of the OVS pulse profile. Two preparation scans were performed at the beginning of each spectral acquisition to achieve steady-state magnetization. Additionally, unsuppressed water spectra were acquired in each subject for eddy current correction (VAPOR on with RF off, OVS on) and another one (VAPOR and OVS off to avoid loss of water signal due to magnetization transfer effects) to use as an internal water concentration reference for metabolite quantification.[19,21] The total scan time for each VOI including shimming, calibration and spectra acquisition was approximately 10 minutes. To evaluate the cerebrospinal fluid (CSF) contribution to VOI, we segmented the brain and estimated the %CSF content in the VOI based on T_1 images as explained below. Metabolite concentrations were then corrected for regional CSF content assuming zero metabolite content in the CSF.

Metabolite quantification

Data pre-processing consisted of eddy current correction and shot-to-shot phase and frequency correction in MATLAB as previously described.[19,21] Summed spectra were quantified with LCModel.[22] The basis set was simulated using density matrix calculation in MATLAB and included: alanine, ascorbate, aspartate, creatine, γ-aminobutyric acid (GABA), glycerophosphorylcholine (GPC), phosphocholine (PCho), phosphocreatine (PCr), glucose (Glc), glutamine (Gln), glutamate (Glu), glutathione (GSH), *myo*-inositol (*myo*-Ins), scyllo-inositol (sIns), lactate, *N*-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphorylethanolamine (PE) and taurine. The basis set also included macromolecule spectra that were acquired in healthy volunteers in a previous study.[19] By using water from the same

VOI as an internal concentration reference, we limited errors that otherwise could arise from the use of an external reference such as those related to B_1 homogeneities and coil loading. Metabolite concentrations were corrected for CSF content and GM/WM partial volume fraction assuming 100% water content in CSF, 78% water content in GM and 65% water content in WM as recommended in the LCModel manual. Even though atrophy is observed in HD, tissue water content seems to remain unchanged in HD.[23] We thus assumed identical tissue water content in GM and WM in controls and patients. In principle, increased CSF fraction should not bias the quantification, since metabolite concentrations are corrected for CSF content. However, T_2 relaxation of water during the semi-LASER protocol could introduce some bias. While we used a short T_E of 28 ms, there is non-negligible relaxation of water especially in tissue, since T_2 of water in tissue (GM and WM) is much shorter than T_2 of water in CSF. If CSF content differs significantly among subjects, a small bias is introduced to the quantification (the more CSF, the less the water reference signal relaxes during TE). We thus corrected the water reference signal intensity for T_2 relaxation during TE using T_2 of water in CSF of 740 ms, and T_2 of water in tissue (GM and WM) to be 100 ms[24] using the formula:

$$S_{CORR} = S_{UNCORR} \times \frac{[\, f_{CSF} + 0.78 \times f_{GM} + 0.65 \times f_{WM} \,]}{\left[\, f_{CSF} \times e^{(-TE/T_{2,CSF})} + 0.78 \times f_{GM} \times e^{(-TE/T_{2,GM})} + 0.65 \times f_{WM} \times e^{(-TE/T_{2,WM})} \right]}$$

Where f_{CSF}, f_{GM} and f_{WM} are fractions of CSF, GM and WM in the voxel and $T_{2,CSF}$, $T_{2,GM}$ and $T_{2,WM}$ are T_2 relaxation times of CSF, GM, and WM.

While the reported T_2 of water in brain tissue are approximately 80 ms, we increased these values by 25% to account for longer T_2 during the Carr-Purcell-Meiboom-Gill train in LASER. The metabolite signal was corrected for relaxation using

T_2(metab,tissue) = 200 ms for all metabolites. While some metabolites have a longer T_2 (e.g. tNAA), the small bias introduced by using the same T_2 would be the same in the control and patient group and would not affect comparisons between the two groups.

Metabolites were considered reliably quantified when Cramér-Rao lower bounds (CRLB) ≤ 20% were observed in at least half the subject population as this threshold allows the selection of the most reliably quantified metabolites as recommended in the LCModel manual. However, in order to avoid quantification bias, we reported the average of all concentration values for each reliably quantified metabolite, including those with CRLB < 999%. For metabolites exhibiting high cross-correlation (correlation coefficient < -0.7) – e.g. Cr and PCr; and NAA and NAAG – only the sum was reported (e.g. tCr for Cr and PCr; tNAA for NAA and NAAG). All spectra had metabolite linewidth < 10 Hz, as estimated by LCModel (average ~5 Hz in visual cortex and ~7-8 Hz in striatum). Pre-processing and quantification steps took approximately 3 minutes per spectrum to complete.

Brain tissue volume estimation

To obtain reliable volume estimates, 3D T_1 images were automatically segmented using Freesurfer v5.3 (https://surfer.nmr.mgh.harvard.edu/). The gray matter (GM)/white matter (WM) interface as well as the GM volume segmentations were examined and reprocessed if corrections were needed. For each subject, VOIs were registered to their respective 3D T1 image and the volume fractions of the GM, WM and cerebrospinal fluid (CSF) within each VOI were extracted and quantified using tools available from Freesurfer.

Statistical analysis

We applied a student *t*-test to compare neurometabolite concentrations between patients and controls. Pearson correlations were performed between neurometabolite concentrations and TMS, CAG repeat length and brain volume fractions in the VOI with Holm-Bonferroni multiple comparison correction. Probability values of $p < 0.05$ were considered significant.

RESULTS

Reduced GM volume fractions and increased CSF fractions were observed in both striatum and visual cortex in HD patients compared to controls (Figure 2). Each subject's volume fractions were used to correct for partial volume effects in determining metabolite concentrations. Correction for CSF content and for T_2 relaxation greatly reduced the SD of metabolite concentrations and minimized sources of bias.

None of the datasets were excluded on the basis of spectral linewidth. We could not acquire one dataset from the striatum of a patient due to scanning time constraints. Hence nine datasets are reported in striatum in patients. None of the metabolites reported in Figure 3 had CRLB = 999 for any subject. In the visual cortex, HD patients displayed a 7% increase in tCr relative to controls ($p < 0.05$) (Figure 3A). All other neurometabolites (Asp, Gln, Glu, GSH, *myo*-Ins, sIns, Tau, tCho and tNAA) had similar concentrations in both groups.

In the striatum, HD patients showed a large 20% decrease in Glu concentration ($p < 0.001$) and a 12% decrease in tCr ($p < 0.05$). All other metabolites were comparable

to controls (Figure 3B). Total NAA showed a 4% trend to decrease in striatum ($p = 0.08$).

In order to examine less concentrated neurometabolites (aspartate, ascorbic acid, *scyllo*-inositol, lactate and taurine), the CRLB threshold was increased to 50%. These additional metabolites with the exception of aspartate in the striatum did not show any significant differences between patients and controls, although glutamine showed a trend to decrease in striatum similar to glutamate (-15%, $p = 0.12$) (Supplementary Figure).

In the visual cortex of patients, tCr correlated positively with CSF fraction ($p < 0.001$), and negatively with GM ($p < 0.05$) and WM ($p < 0.001$) volume fractions (Figure 4A) but not with TMS, CAG repeat length or age. In the striatum of patients, Glu correlated negatively with CSF fraction ($p < 0.05$) and positively with WM volume fraction ($p < 0.05$) (Figure 4B) but not with TMS, CAG repeat length or age.

DISCUSSION

We showed increased tCr in the visual cortex and decreased Glu and tCr in the striatum at an early stage of HD, but no significant changes in less prominent metabolites such as GABA, Gln or GSH. While the striatum is at the center of structural and metabolic changes in HD, we confirmed that the visual cortex is also involved in the early stage of the disease at both structural and metabolic levels. The extended analysis of 10 neurometabolites in the visual cortex and 8 in the striatum was possible due to a previously validated semi-LASER sequence providing high SNR, small chemical shift displacement and minimal *J*-modulation. The lower

number of metabolites reported for the striatum compared to visual cortex is due in part to broader linewidth (metabolite linewidth ~7-8 Hz in striatum compared to ~5 Hz in visual cortex). In contrast, GABA was quantified with CRLB < 20% in striatum but not in visual cortex due to its two-fold higher concentration in striatum. Metabolites of low concentration such as Asp, GSH, sIns and Tau are often very difficult to quantify at 3 T due to their small resonance peaks. Hence, changes in the baseline could influence their quantification even when highly resolved metabolite peaks with excellent spectral quality are achieved.

Most clinical protocols use PRESS and STEAM sequences, which are vendor provided sequences. In the previous studies compiled in Table 1, long T_E protocols[25,26] allowed only a limited number of metabolites to be reported since J-coupled and shorter T_2 metabolites could not be measured. Other studies used short T_E STEAM[12,15,27] and PRESS[14,28,29] protocols to report "absolute" (i.e. water-referenced) concentrations or ratios of metabolites. However, STEAM produces spectra with ~two-fold lower SNR and PRESS suffers from high chemical shift displacement error.[30] In semi-LASER, the two pairs of 180° AFP pulses minimize *J*-modulation and allow measurement of *J*-coupled as well as singlet metabolites with optimal sensitivity.[19]

Cr and PCr whose summed resonance peak is referred to as total creatine (tCr), serve as an energetic marker and maintains brain energy homeostasis.[31] The importance of tCr may stem from the limited glucose storage capability by the brain.[32] Increased tCr has been reported in the brain of HD mouse models[33] and this increase preceded the depletion of ATP.[34] Patients with spinocerebellar ataxia type 1, 2 and 3, another group of polyglutamine disorders that share common pathophysiological pathways with HD,[35] also displayed increased tCr in the vermis and the pons.[21] In addition, the

relative concentrations of components of energy metabolism – ATP, PCr and inorganic phosphate (Pi) – were previously measured using ^{31}P MRS in the visual cortex of HD patients before, during and after visual stimulation.[7,8] Healthy controls exhibited a normal profile, with increased Pi/PCr ratio during visual stimulation, followed by a return to baseline levels during recovery. In contrast, HD patients displayed no significant change in Pi/PCr ratio in two independent studies.[7,8] The higher tCr in the visual cortex reported here is in agreement with these studies. Altogether, these studies underlie that creatine metabolism is modified in the visual cortex of HD patients, a region that is particularly enriched in mitochondria,[7,8] at an early stage of the disease and may signify a compensatory mechanism. In the striatum however, we observed a decrease in tCr concentration which is in line with some previous studies.[12,14,15,36] This decrease might point to a failure to initiate energetic compensatory mechanisms as we have observed in the visual cortex. It should be noted that the p values reported for tCr and Glu were not corrected for multiple comparisons within groups. It is therefore possible that our observations on tCr, with p-values close to statistical significance, might more likely be a trend to increase. Our interpretation of tCr changes must therefore be taken with caution also considering the small sample size.

The significant decrease in the neuronal marker Glu in the striatum is in agreement with previous reports from the putamen.[14,15] Decreased Glu could be due in part to the lower GM content in the striatal VOI. However, assuming that Glu was two-fold higher in GM than in WM,[37] we calculated that the measured decrease in GM content would have resulted in a less than 5% decrease in Glu concentration in the VOI, and therefore did not explain the 20% decrease observed in our study. Since it has been shown that the striatum is highly affected in HD, even at the presymptomatic stage,[2]

decreased Glu concentrations could reflect neuronal dysfunction as a result of atrophy. Furthermore, Glu levels have been linked to metabolic activity.[31] Twenty percent of brain glucose metabolism is directed to Glu synthesis through the Gln-Glu cycle.[38] As HD patients display glucose hypometabolism in the striatum and cortex,[39] decreased Glu levels could reflect decreased metabolic activity in HD patients.

Consistent with several other studies reported in Table 1 which reported changes in tNAA, we observed a trend in decrease in tNAA in striatum (from 9.0 ± 0.4 to 8.6 ± 0.5 mmol/g), although it did not reach statistical significance ($p = 0.08$). This could be due to the fact that our cohort was at a very early stage of the disease or that we had a relatively small sample size. Some previous studies reported no change in tNAA but a decrease in NAA.[12,14,15,36] In fact, in our study, NAA was significantly decreased in the striatum (from 8.6 ± 0.3 to 7.8 ± 0.5 mM, $p < 0.001$), but this decrease was partly compensated by an increase in NAAG (from 0.4 ± 0.3 to 0.8 ± 0.4 mM, $p < 0.02$). However, individual NAA and NAAG concentrations are generally considered unreliable at 3 Tesla due to their high cross-correlation, and we consider that only the sum tNAA can be quantified reliably.

GABA is another important metabolite in HD since the medium-sized spiny neurons that contain GABA undergo selective degeneration in the striatum.[40] Although spectral editing is often performed to quantify GABA by MRS, GABA can also be quantified from high-quality short-TE ^{1}H spectra such as obtained with LASER or semi-LASER, especially in brain regions that have higher GABA concentration.[41] Here GABA was quantified with good precision in striatum due to its higher concentration (~2.2 - 2.4 μmol/g), but not in visual cortex (~1 μmol/g). One previous study reported GABA in striatum in HD at 7 Tesla and also reported no change.[42]

Reports of changes in *myo*-Ins and tCho in striatum in early HD are mixed. Some studies showed increased *myo*-Ins and/or tCho, but some did not (see Table 1). Our study showed no evidence of changes in tCho or *myo*-Ins in striatum, in agreement with the only reported measurements in striatum in HD at 7 Tesla,[15] a higher magnetic field expected to provide improved accuracy. Our study is also consistent with the main findings of that study[15], namely lower Glu, lower tCr and lower tNAA (or in our case trend to lower tNAA) in striatum. The main evidence for increased *myo*-Ins in striatum in early HD is from Sturrock et al.[14] However, in that study, spectra from early HD patients appear much broader than spectra from controls and pre-HD patients, and the degraded spectral quality could conceivably affect *myo*-Ins and tCho concentrations determined by LCModel. Some earlier studies reported increased tCho based on increased tCho/tCr ratio; however, more recent studies using water as a concentration reference suggest that the increased ratio actually results from decreased tCr rather than increased tCho.

In conclusion, this study used an optimized semi-LASER protocol to help understand the early neurometabolic alterations that occur in HD. We reported absolute metabolite concentrations and not ratios,[11,25,29,36,43] in order to be able to analyse the impact of each individual metabolite. Unlike all the aforementioned studies summarized in Table 1, our semi-LASER protocol allowed to obtain an expanded neurochemical profile of up to 10 metabolites, which is promising to find robust biomarkers of HD.

Acknowledgements

The authors wish to thank warmly the patients and volunteers who participated in this study. The authors are also grateful to collaborators from the Center for Neuroimaging Research, France, and the Center for Magnetic Resonance Research, USA.

DISCLOSURE/CONFLICT OF INTEREST

No disclosure: Adanyeguh, Monin, Rinaldi.

Freeman: Research support by grants from the National Multiple Sclerosis Society.

Durr: Research support by grants from the French Agency for Research, Fondation pour la recherche médicale (FRM), and Pfizer Inc.

Lehericy: Received grants from Agence Nationale de la Recherche (ANRMNP 2009, Nucleipark), DHOS-Inserm (2010, Nucleipark), France Parkinson (2008), Ecole Neuroscience de Paris, 'Investissements d'avenir' [grant number ANR-10-IAIHU-06 and ANR-11-INBS-0006] during the conduct of the study. Outside of this study, he received commercial research support from Servier and Pfizer, funding for travel from Siemens and General Electric and honoraria from Pileje, Lundbeck and Roche.

Henry: Research support by grants from NIH (P41 EB015894, P30 NS076408), Friedreich's Ataxia Research Alliance and CureFA foundation.

Mochel: Research support by grants from INSERM, Carnot Institutes, ASL Foundation and Ultragenyx Pharmaceutical.

REFERENCES

1. Kremer B, Goldberg P, Andrew SE, et al. A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. N Engl J Med 1994;330(20):1401-1406.

2. Tabrizi SJ, Scahill RI, Owen G, et al. Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. Lancet Neurol 2013;12:637-649.

3. Rosas HD, Koroshetz WJ, Chen YI, et al. Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. Neurology 2003;60:1615-1620.

4. Waldvogel HJ, Kim EH, Tippett LJ, Vonsattel JP, Faull RL. The Neuropathology of Huntington's Disease. Curr Top Behav Neurosci 2015;22:33-80.

5. Rub U, Seidel K, Vonsattel JP, et al. Huntington's Disease (HD): Neurodegeneration of Brodmann's Primary Visual Area 17 (BA17). Brain Pathol 2015;25(6):701-711.

6. Feigin A, Leenders KL, Moeller JR, et al. Metabolic network abnormalities in early Huntington's disease: an [(18)F]FDG PET study. J Nucl Med 2001;42(11):1591-1595.

7. Mochel F, N'Guyen TM, Deelchand D, et al. Abnormal response to cortical activation in early stages of Huntington disease. Mov Disord 2012;27:907-910.

8. Adanyeguh IM, Rinaldi D, Henry PG, et al. Triheptanoin improves brain energy metabolism in patients with Huntington disease. Neurology 2015;84:490-495.

9. Corvol JC. Neuroprevention: a new challenge? Rev Neurol (Paris) 2012;168:796-801.

10. Huntington Study Group. Unified Huntington's disease rating scale: Reliability and consistency. Mov Disord 1996;11:136-142.

11. Jenkins BG, Rosas HD, Chen YC, et al. 1H NMR spectroscopy studies of Huntington's disease: correlations with CAG repeat numbers. Neurology 1998;50:1357-1365.

12. Clarke CE, Lowry M, Quarrell OWJ. No change in striatal glutamate in Huntington's disease measured by proton magnetic resonance spectroscopy. Parkinsonism Relat Disord 1998;4:123-127.

13. Ruocco HH, Lopes-Cendes I, Li LM, Cendes F. Evidence of thalamic dysfunction in Huntington disease by proton magnetic resonance spectroscopy. Mov Disord 2007;22:2052-2056.

14. Sturrock A, Laule C, Decolongon J, et al. Magnetic resonance spectroscopy biomarkers in premanifest and early Huntington disease. Neurology 2010;75:1702-1710.

15. van den Bogaard SJ, Dumas EM, Teeuwisse WM, et al. Exploratory 7-Tesla magnetic resonance spectroscopy in Huntington's disease provides in vivo evidence for impaired energy metabolism. J Neurol 2011;258:2230-2239.

16. Liot G, Valette J, Pépin J, Flament J, Brouillet E. Energy defects in Huntington's disease: Why "in vivo" evidence matters. Biochem Biophys Res Commun 2017;483:1084-1095.

17. Tkác I, Öz G, Adriany G, et al. In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs. 7T. Magn Reson Med 2009;62:868-879.

18. Öz G, Tkáč I. Short-echo, single-shot, full-intensity proton magnetic resonance spectroscopy for neurochemical profiling at 4 T: Validation in the cerebellum and brainstem. Magn Reson Med 2011;65:901-910.

19. Deelchand DK, Adanyeguh IM, Emir UE, et al. Two-site reproducibility of cerebellar and brainstem neurochemical profiles with short-echo, single-voxel MRS at 3T. Magn Reson Med 2014;73:1718-1725.

20. Gruetter R, Tkac I. Field mapping without reference scan using asymmetric echo-planar techniques. Magn Reson Med 2000;43(2):319-323.

21. Adanyeguh IM, Henry PG, Nguyen TM, et al. In vivo neurometabolic profiling in patients with spinocerebellar ataxia types 1, 2, 3, and 7. Mov Disord 2015;30:662-670.

22. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 1993;30:672-679.

23. Zacharoff L, Tkac I, Song Q, et al. Cortical metabolites as biomarkers in the R6/2 model of Huntington's disease. J Cereb Blood Flow Metab 2012;32:503-514.

24. Zhou J, Golay X, van Zijl PC, et al. Inverse T(2) contrast at 1.5 Tesla between gray matter and white matter in the occipital lobe of normal adult human brain. Magn Reson Med 2001;46:401-406.

25. Taylor-Robinson SD, Weeks RA, Bryant DJ, et al. Proton magnetic resonance spectroscopy in Huntington's disease: evidence in favour of the glutamate excitotoxic theory. Mov Disord 1996;11:167-173.

26. van Oostrom JC, Sijens PE, Roos RA and Leenders KL. 1H magnetic resonance spectroscopy in preclinical Huntington disease. Brain Res 2007;1168:67-71.

27. van den Bogaard SJ, Dumas EM, Teeuwisse WM, et al. Longitudinal metabolite changes in Huntington's disease during disease onset. J Huntingtons Dis 2014;3:377-386.

28. Sturrock A, Laule C, Wyper K, et al. A longitudinal study of magnetic resonance spectroscopy Huntington's disease biomarkers. Mov Disord 2015;30:393-401.

29. Padowski JM, Weaver KE, Richards TL, et al. Neurochemical correlates of caudate atrophy in Huntington's disease. Mov Disord 2014;29:327-335.

30. Boer VO, van Lier AL, Hoogduin JM, Wijnen JP, Luijten PR, Klomp DW. 7-T 1H MRS with adiabatic refocusing at short TE using radiofrequency focusing with a dual-channel volume transmit coil. NMR Biomed 2011;24:1038-1046

31. Rae CD. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. Neurochem Res 2014;39:1-36.

32. Brewer GJ, Wallimann TW. Protective effect of the energy precursor creatine against toxicity of glutamate and beta-amyloid in rat hippocampal neurons. J Neurochem 2000;74:1968-1978.

33. Tkac I, Dubinsky JM, Keene CD, et al. Neurochemical changes in Huntington R6/2 mouse striatum detected by in vivo 1H NMR spectroscopy. J Neurochem 2007;100:1397-1406.

34. Mochel F, Durant B, Meng X, et al. Early alterations of brain cellular energy homeostasis in Huntington disease models. J Biol Chem 2012;287:1361-1370.

35. Gatchel JR and Zoghbi HY. Diseases of unstable repeat expansion: mechanisms and common principles. Nat Rev Genet 2005;6:743-755.

36. Hoang TQ, Bluml S, Dubowitz DJ, et al. Quantitative proton-decoupled 31P MRS and 1H MRS in the evaluation of Huntington's and Parkinson's diseases. Neurology 1998;50:1033-1040.

37. Hassel B, Dingledine R. Glutamate. In: Siegel GJ, Albers RW, Brady S, Price D, editors. Basic neurochemistry: molecular, cellular and medical aspects. 7th edition. San Diego: Elsevier Academic Press; 2005:267-290.

38. Maddock RJ, Buonocore MH. MR spectroscopic studies of the brain in psychiatric disorders. Curr Topics Behav Neurosci 2012;11:199-251.

39. Pagano G, Niccolini F, Politis M. Current status of PET imaging in Huntington's disease. Eur J Nucl Med Mol Imaging 2016;43:1171-1182.

40. Vonsattel JP, DiFiglia M. Huntington disease. J Neuropathol Exp Neurol 1998;57(5):369-384.

41. Oz G, Terpstra M, Tkác I, et al. Proton MRS of the unilateral substantia nigra in the human brain at 4 tesla: detection of high GABA concentrations. Magn Reson Med 2006;55:296-301.

42. Unschuld PG, Edden RA, Carass A, et al. Brain metabolite alterations and cognitive dysfunction in early Huntington's disease.Mov Disord 2012;27:895-902.

43. Jenkins BG, Koroshetz WJ, Beal MF, Rosen BR. Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized 1H NMR spectroscopy. Neurology 1993;43:2689-2695.

44. Gómez-Ansón B, Alegret M, Muñoz E, et al. Decreased frontal choline and neuropsychological performance in preclinical Huntington disease. Neurology 2007;68:906-910.

45. Sánchez-Pernaute R, García-Segura JM, del Barrio Alba A, et al. Clinical correlation of striatal 1H MRS changes in Huntington's disease. Neurology 1999;53:806-812.

Table 1: Summary of previous ^{1}H MRS studies in the striatum and occipital cortex in HD.

	Striatum	Caudate	Putamen	Occipital cortex
Presymptomatic vs controls				
NAA/tNAA			↘ NAA -8% but not tNAA Sturrock 2010 (n=25)[14] No change in NAA but ↘tNAA Sturrock 2015 (n=25)[28]	
No change in any metabolite	Gomez-Anson 2007 (n=19)[44]	Van den Bogaard 2011 (n=11 reported)[15]	Van den Bogaard 2011 (n=9 reported)[15] Van Oostrom 2007 (n=19)[2]	
Patients vs controls				
NAA/tNAA	↘ Jenkins 1993 (tNAA/Cr, n=15)[43] ↘ Jenkins 1998 (tNAA/Cr, n=31)[11] ↘ Clarke 1998 (NAA, n=6)[12]	↘ Van den Bogaard 2011 (n=5 reported)[15]	↘ -17%/-15% Sturrock 2010 (n=29)[14] ↘ Van den Bogaard 2011 (n=5 reported)[15] ↘ NAA Hoang 1998 (n=15)[36] ↘ Sturrock 2015 (n=23/24)[28]	↘ NAA/Cr Hoang 1998[35]
mIns			↗ Hoang 1998 (n=15)[36] ↗ ~50% Sturrock 2010 (n=29)[14] ↗ Sturrock 2015 (n=23/24)[28]	
tCr	↘ Clarke 1998 (Cr) (n=6)[12]	↘ Van den Bogaard 2011 (n=5 reported)[15]	↘ Van den Bogaard 2011 (n=5 reported)[15] ↘ Hoang 1998 (n=15)[36] ↘ -18% Sturrock 2010 (n=29)[14]	
Glu	↗ Glx Taylor-Robinson 1996 (Glx/tCr, n=5)[25]		↘ Van den Bogaard 2011 (n=5 reported)[15] ↘ ~10% Sturrock 2010 (n=29)[14]	
Cho/tCho	↗ Jenkins 1993 (tCho/tCr, n=15)[44] ↗ Jenkins 1998 (tCho/tCr, n=31)[11] ↗ Clarke 1998 (Cho/Cr, n=6)[12]		↗ ~10% Sturrock 2010 (n=29)[14] ↗ Hoang 1998 (n=15)[36]	↗ Jenkins 1998 (n=31)[11]
Lac	↗ Jenkins 1993 (n=15)[43] ↗ Jenkins 1998 (n=31)[11]			↗ Jenkins 1993 (n=15)[43] ↗ Jenkins 1998 (n=31)[11]
No change in any metabolite				Taylor-Robinson 1996[25] Hoang 1998 (abs conc)[36]
Mixed presymptomatic/patients vs controls				
NAA/tNAA		↘ Sanchez-Pernaute 1999 (n=4 PMC, n=6 MC)[45]		
mIns			Trend ↗ Padowski 2014 (n=6 PMC, n=4 MC)[29]	
tCr		↘ Sanchez-Pernaute 1999 (n=4 PMC, n=6 MC)[45]		
Glu		↘ Padowski 2014 (only when considering ratios) (n=6 PMC, n=4 MC)[29]		
Mixed presymptomatic/patients longitudinal changes				
NAA/tNAA			↘ van den Bogaard 2014 (n=6 PMC, n=1 MC)[27]	
mIns		↘ van den Bogaard 2014 (n=7 PMC, n=2 MC)[27]	Trend mIns/NAA ↗ Sturrock 2015 (n = 23/24 MC)[28]	
Cr		↘ van den Bogaard 2014 (n=7 PMC, n=2 MC)[27]		
Cho			↘ van den Bogaard 2014 (n=6 PMC, n=1 MC)[27]	
No change in any metabolite			Sturrock 2015 (n=25/22 PMC, n= 23/24 MC)[28]	

NAA: *N*-acetylaspartate, tNAA: total *N*-acetylaspartate. Cr: creatine, tCr: total creatine, Glu: glutamate, Glx: glutamine and glutamate, tCho: total choline, Lac: lactate, *myo*-Ins: *myo*-inositol, PMC: premanifest carrier; MC: manifest carrier, abs conc: absolute concentration; ↗: increased concentration; ↘: decreased concentration

Table 2: Demographic and spectroscopic parameters of participants.

Variable	Controls	HD patients
Nº of participants	10.0	10.0
Gender (M/F)	3/7	3/7
Age (years)	38.9 ± 13.8	45.6 ± 12.7
BMI (kg/m^2)	21.1 ± 1.7	21.6 ± 3.3
TMS (UHDRS)	0.9 ± 1.0	13.6 ± 10.8 $^{\#}$
CAG length		44.1 ± 4.2
Lw striatum (Hz)	6.7 ± 1.0	8.4 ± 1.2 *
Lw visual cortex (Hz)	4.6 ± 0.5	4.6 ± 0.5
SNR striatum	46.7 ± 11.3	39.0 ± 16.7
SNR visual cortex	65.6 ± 19.4	57.1 ± 14.5
%CSF striatum	4.5 ± 1.4	12.0 ± 8.4 *
%CSF visual cortex	7.0 ± 4.8	17.0 ± 11.5 *

Data are presented as mean ± standard deviation and compared by *t*-test. BMI: body mass index; TMS: total motor score; SNR: signal-to-noise ratio estimated by LCModel; Lw: water linewidth estimated by LCModel; CSF: cerebrospinal fluid. *$p<0.05$ and $^{\#}p\leq0.01$ represent significant differences between HD patients and controls.

FIGURE LEGENDS

Figure 1: Voxel positioning, spectra quality and model fitting by LCModel in the visual cortex and striatum. Spectra were acquired in an acquisition voxel of 25 x 25 x 25 mm^3 in the visual cortex and 34 x 19 x 23 mm^3 in the striatum using the modified semi-LASER sequence (T_R = 5000 ms, T_E = 28 ms, averages = 64). The black lines are the raw spectra, whilst the red lines are the LCModel fits.

Figure 2: Brain tissue volume fraction in VOI in the striatum and visual cortex. Gray matter (GM) was markedly reduced in the striatum and visual cortex whilst cerebrospinal fluid (CSF) was significantly increased. *$p<0.05$ and #$p\leq0.01$ represent significant differences between HD patients and controls.

Figure 3: Mean metabolite concentrations obtained in the A) visual cortex and B) striatum. Fewer metabolites are reported for the striatum since they did not meet the quality control threshold unlike in the visual cortex. Asp: aspartate, Gln: glutamine, Glu: glutamate, GSH: glutathione, myo-Ins: *myo*-inositol, sIns: *scyllo*-inositol, Tau: taurine, tCho: total choline, tCr: total creatine, tNAA: total *N*-acetylaspartate. Error bars represent standard deviations. *$p< 0.05$, †$p\leq 0.001$.

Figure 4: Neurometabolite correlations with brain volume fractions of patients. A) tCr correlated positively with CSF fraction ($p = 0.001$), and negatively with GM ($p < 0.05$) and WM ($p < 0.001$) volume fractions in the visual cortex. B) Glu correlated negatively with CSF fraction ($p < 0.05$) and positively with WM volume fraction ($p < 0.05$) in the striatum.