Response document

Reviewer 00646357

-Add about other methods of analysis of chemical shift imaging

R: I thank the reviewer for this suggestion. I have added more details on chemical shift imaging, as follows. "Thus, in neuroimaging, 2D-CSI has the distinct advantage over SVS of allowing larger areas of the brain to be studied during scanning, so that areas showing abnormal signals and also those appearing normal in structural magnetic resonance images can be included [2]. CSI can also be carried out in three dimensions, which should improve spatial resolution and the signal-to-noise ratio; however, 2D-CSI is more resistant to motion artefact, which can be a problem when scanning the brain, than 3D-CSI [3]. Furthermore, image quality is better with 2D-CSI compared with 3D-CSI at a usual magnetic field strength of 1.5 T or 3 T [4-6]."

-More about choline and its clinical value

R: I thank the reviewer for this suggestion. I have added more details on choline and its clinical value, as follows. "Choline is an alcohol which, in the human brain, is particularly abundant in phosphatidylcholine (in which it is attached, as a polar head group, via a phosphate group, to the Sn3 position of the glycerol backbone) membrane phospholipid molecules; Cho take part in membrane biosynthesis and breakdown [1]. Thus, measurement of Cho has clinical and research value. One example is in relation to chronic fatigue syndrome (also known as myalgic encephalomyelitis or systemic exertion intolerance disease), which is currently of unknown aetiology. The first systematic proton neurospectroscopy study of this condition showed a significantly higher level of Cho in the occipital cortex in patients compared with matched healthy controls, and also loss of the spatial variation of Cho that is normally expected [7]. Given that such increased levels are associated with abnormal membrane phospholipid metabolism [8], this finding, which was essentially confirmed later by another group in respect of the basal ganglia [9], suggests that chronic fatigue syndrome/myalgic encephalomyelitis is associated with abnormal phospholipid metabolism in neuroglial membranes [1, 7]. It has been suggested that this, in turn, might result from chronic viral infection [10]. Based on this Cho finding, a potential therapeutic approach to this difficult-to-treat disorder, involving long-chain polyunsaturated fatty acids, has been suggested [11, 12]. A second example relates to dyslexia, which is another important neuropsychiatric disorder of unknown aetiology, in which the first systematic proton neurospectroscopy study revealed decreased Cho in the left temporo-parietal lobe [13]. This finding could have resulted from reduced left temporo-parietal phospholipid metabolism [14], which would be consistent with the findings from the first systematic 31-phosphorus neurospectroscopy study of this disorder [15]. In turn, this has led to suggestions of potential therapeutic interventions [16]."

-More about analysis, one slide or more, who do analysis, one or more and their experience and inter-observer analysis.

R: The statistical analyses were carried out by me (BKP). This is already mentioned on the first page, under "Author contributions". The analysis of each spectrum was automated and carried out by the software in the Siemens spectroscopy task card. This is already mentioned in the Methods section, in the sentence "Spectral analysis was carried out using the Siemens spectroscopy task card (Siemens Medical Systems, Erlangen, Germany)." Since multiple observers were not involved, clearly no inter-observer analysis was required. It is clear that the reviewer wishes this to be further clarified and emphasised in the paper. I agree with this and thank the reviewer for this suggestion. Accordingly, I have added the following sentence of clarification to the Methods section: "This automated software analysis was objective and clearly obviated the need for inter-observer analysis."

-Update of references

R: I thank the reviewer for this suggestion and I have updated the references, with the inclusion of the following new ones.

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4 **Fischer MA**, Donati OF, Chuck N, Blume IN, Hunziker R, Alkadhi H, Nanz D. Two- versus three-dimensional dual gradient-echo MRI of the liver: a technical comparison. *Eur Radiol.* 2013;**23**:408-416. [PMID: 22865276 DOI: 10.1007/s00330-012-2614-z]

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7 **Puri BK**, Counsell SJ, Zaman R, Main J, Collins AG, Hajnal JV, Davey NJ. Relative increase in choline in the occipital cortex in chronic fatigue syndrome. *Acta Psychiatr Scand.* 2002;**106**:224-226. [PMID: 12197861]

8 **Ruiz-Cabello J**, Cohen JS. Phospholipid metabolites as indicators of cancer cell function. *NMR in Biomedicine.* 1992;**5**:226-233. [DOI: 10.1002/nbm.1940050506]

9 **Chaudhuri A**, Condon BR, Gow JW, Brennan D, Hadley DM. Proton magnetic resonance spectroscopy of basal ganglia in chronic fatigue syndrome. *Neuroreport.* 2003;**14**:225-228. [PMID: 12598734 DOI: 10.1097/01.wnr.0000054960.21656.64]

10 **Puri BK**. Long-chain polyunsaturated fatty acids and the pathophysiology of myalgic encephalomyelitis (chronic fatigue syndrome). *J Clin Pathol.* 2007;**60**:122-124. [PMID: 16935966 PMCID: PMC1860620 DOI: 10.1136/jcp.2006.042424]

11 **Puri BK**, Holmes J, Hamilton G. Eicosapentaenoic acid-rich essential fatty acid supplementation in chronic fatigue syndrome associated with symptom remission and structural brain changes. *Int J Clin Pract.* 2004;**58**:297-299. [PMID: 15117099]

12 **Puri BK**. The use of eicosapentaenoic acid in the treatment of chronic fatigue syndrome. *Prostaglandins Leukot Essent Fatty Acids.* 2004;**70**:399-401. [PMID: 15041033 DOI: 10.1016/j.plefa.2003.12.015]

13 **Rae C**, Lee MA, Dixon RM, Blamire AM, Thompson CH, Styles P, Talcott J, Richardson AJ, Stein JF. Metabolic abnormalities in developmental dyslexia detected by 1H magnetic resonance spectroscopy. *The Lancet.* 1998;**351**:1849-1852. [DOI: https://doi.org/10.1016/S0140-6736(97)99001-2]

14 **Puri BK**, Richardson AJ. Brain phospholipid metabolism in dyslexia assessed by magnetic resonance spectroscopy. In: Peet M, Glen AI, Horrobin DF, editors. Phospholipid Spectrum Disorders in Psychiatry and Neurology. 2nd edition ed. Carnforth Lancashire: Marius Press, 2003: 501-508

15 **Richardson AJ**, Cox IJ, Sargentoni J, Puri BK. Abnormal cerebral phospholipid metabolism in dyslexia indicated by phosphorus-31 magnetic resonance spectroscopy. *NMR Biomed.* 1997;**10**:309-314. [PMID: 9471121]

16 **Puri BK**. Proton and 31-phosphorus neurospectroscopy in the study of membrane phospholipids and fatty acid intervention in schizophrenia, depression, chronic fatigue syndrome (myalgic encephalomyelitis) and dyslexia. *Int Rev Psychiatry.* 2006;**18**:145-147. [PMID: 16777668 DOI: 10.1080/09540260600581852]

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29 **Puri BK**. Neurospectroscopy. In: Puri BK, Treasaden IH, editors. Forensic Psychiatry: Fundamentals and Clinical Practice. Boca Raton, Florida, USA: CRC Press, 2017: 37-38

Reviewer 02445209

Dear authors, I only have a few comments on your manuscript, which is otherwise excellent

I thank the reviewer for this kind assessment.

- The last paragraph of Discussion: You use the term "bleeding" in the neurospectroscopy data acquisition. Would you explain this in more detail to a reader?

R: I thank the reviewer for this suggestion. I have added the following clarification to the last paragraph of the Discussion. "Voxel "bleeding" refers to contamination with signals derived from any of the six adjacent voxels, and is an analogue of artifactual Gibbs ringing in structural MRI [27]. In the present case, the contaminating signals could have arisen from the low-signal sulcal spaces."

- Conclusion: Would you suggest more specific areas in psychiatric clinical practice, where the described technique could be applied (in which diseases, whether in diagnostics, treatment, prevention etc.)?

R: I thank the reviewer for this suggestion. I have added the following to the Conclusion. "Already, neurospectroscopy is proving helpful in studies of schizophrenia, major depressive disorder, forensic psychiatry (e.g. posttraumatic stress disorder), chronic fatigue syndrome (myalgic encephalomyelitis or systemic exertion intolerance disease), and neuropsychiatric presentations in organic disorders, in which it has an important role to play in aiding diagnosis [16, 28, 29]. Given the present finding of a highly acceptable level of reproducibility of 2D-CSI, it would be appropriate in future to apply this technique to the follow-up of such patients, including monitoring their response to treatment." In addition, further details of neuropsychiatric applications now also appear in the Introduction, as follows. "Thus, measurement of Cho has clinical and research value. One example is in relation to chronic fatigue syndrome (also known as myalgic encephalomyelitis or systemic exertion intolerance disease), which is currently of unknown aetiology. The first systematic proton neurospectroscopy study of this condition showed a significantly higher level of Cho in the occipital cortex in patients compared with matched healthy controls, and also loss of the spatial variation of Cho that is normally expected [7]. Given that such increased levels are associated with abnormal membrane phospholipid metabolism [8], this finding, which was essentially confirmed later by another group in respect of the basal ganglia [9], suggests that chronic fatigue syndrome/myalgic encephalomyelitis is associated with abnormal

phospholipid metabolism in neuroglial membranes [1, 7]. It has been suggested that this, in turn, might result from chronic viral infection [10]. Based on this Cho finding, a potential therapeutic approach to this difficult-to-treat disorder, involving long-chain polyunsaturated fatty acids, has been suggested [11, 12]. A second example relates to dyslexia, which is another important neuropsychiatric disorder of unknown aetiology, in which the first systematic proton neurospectroscopy study revealed decreased Cho in the left temporo-parietal lobe [13]. This finding could have resulted from reduced left temporo-parietal phospholipid metabolism [14], which would be consistent with the findings from the first systematic 31-phosphorus neurospectroscopy study of this disorder [15]. In turn, this has led to suggestions of potential therapeutic interventions [16].” There are also further details in the following paragraph of the Introduction: “2D-CSI may also be useful clinically in evaluating patients with acute onset of neuropsychiatric systemic lupus erythematosus [2]. Another important clinical use of 2D-CSI is in relation to grading gliomas when used in combination with diffusion kurtosis imaging and dynamic susceptibility-weighted contrast-enhanced MRI [17]. Indeed, in a brain histopathological study, it has been shown that 2D-CSI combined with perfusion MRI are associated with high sensibility and high specificity in differentiating between glioblastoma multiforme and cerebral metastases and also in distinguishing between grade III and grade IV gliomas [18].”

- Table 1 and Table 2 are too extensive, is it not possible to make it shorter?

R: I thank the reviewer for this suggestion. I have shortened both Table 1 and Table 2 by removing reference, in the final column of each table, to “*P* =” and “*P* <” and instead adding the term “(*P* value)” to the end of the header of the final column of both Table 1 and Table 2.

I should like to take this opportunity to thank both reviewers for their very helpful comments.