

High-resolution magnetic resonance imaging sinc-interpolation-based subvoxel registration and semi-automated quantitative lateral ventricular morphology employing threshold computation and binary image creation in the study of fatty acid interventions in schizophrenia, depression, chronic fatigue syndrome and Huntington's disease

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Summary

Serial high-resolution structural magnetic resonance imaging scans of the brain can now be precisely aligned, with six degrees of freedom (three mutually orthogonal translational and three rotational degrees of freedom around three mutually orthogonal axes), using a rigid-body subvoxel registration technique. This is driven by the in-plane point spread function for images acquired in the Fourier domain with data obtained over a bounded region of k -space, namely the sinc interpolation function, where $\text{sinc } z = (\sin z)/z$, with z being any complex number (including zero). Computational subtraction of the three-dimensional Cartesian spatial representation matrices of serially acquired scan data allows for the determination of structural cerebral changes with great precision, since voxel signals from unchanged structures are almost completely cancelled. Thus changes readily show up against a background of noise. Furthermore, lateral ventricular changes can now be accurately quantified using a semi-automated method involving contour production, threshold computation, binary image creation and ventricular extraction. These techniques have been applied to the investigation of the effects on cerebral structure of intervention with fatty acids, particularly the long-chain polyunsaturated n-3 fatty acid eicosapentaenoic acid (EPA), in disorders such as schizophrenia, treatment-resistant depression, chronic fatigue syndrome (myalgic encephalomyelitis or ME), and Huntington's disease.

Introduction

In this paper I review the application to fatty acid research in neurology and psychiatry of two relatively new magnetic resonance imaging (MRI) techniques; my colleagues at Hammersmith Hospital and I have been involved in the development of these techniques. The first method allows the three-dimensional Cartesian spatial representation matrices of two or more serially acquired brain scans from the same subject to be precisely aligned, following which one matrix (usually chosen to be the one based on the baseline scanning data) can readily be subtracted from others. This monomodal rigid-body registration and subtraction technique leads to the almost complete cancellation of signals in voxels (three-dimensional pixels) that correspond to cerebral structures that have not changed between scans. Hence, even very small changes between serial scans show up against a background of noise. Moreover, since the technique aligns the images with a high degree of accuracy, corresponding to a precision that

is subvoxel, artefacts, such as trans-planar shifts associated with traditional methods of naked-eye radiographic alignment, caused by the fact that it is virtually impossible to ensure that the brain is in exactly the same position with respect to the magnetic field during two different scanning sessions, are eliminated. The second technique is a semi-automated procedure to quantify ventricular volumes from T_1 -weighted volume MR scans.

After describing these two methods, their application to studying the effects on cerebral structure of fatty acid intervention is described.

Detection of brain changes using subvoxel registration and subtraction

In order to describe the theory underlying this technique, it is necessary to distinguish between the resolution of an image of an object, and the information contained in that image relating to its position in three-dimensional space.

The resolution is a measure of the smallest feature that can be resolved. For most MR images the resolution is given by the quotient of the field of view and the number of phase- (or frequency-) encoded steps used to acquire the image. The linear dimensions of the image voxels are usually equal to a resolution element. If the data are zero-filled before image reconstruction, then these linear dimensions are slightly smaller than a resolution element.

In contrast, positional information relates to the precision with which the position of an object can be located within an image. It is determined by the distribution of voxel intensity values. Differences in object position that are much less than the dimension of a voxel (that is, subvoxel shifts) can be detected on serial images. A difference image from two serial images can be obtained by subtracting the baseline image from the second image.

Changes in image voxel signal intensities resulting from a subvoxel shift of an object are greatest at the boundaries and least in the central high signal intensity region of the object. In order to distinguish between genuine differences in the object over successive scans, and misregistration effects, it is necessary to carry out positional matching of the images to a small fraction of a resolution element. For example, Hajnal et al. (1995) achieved a match that was accurate to substantially less than one-thirty-ninth of a resolution element, which meant that the image data had to be interpolated to generate new intensity values on the fixed image pixel grid.

With respect to neuroimaging, in practice difference signals must be detected against a background of image noise and artefacts. In the images of the brain acquired by Hajnal and Bydder (1997), the signal-to-noise ratio for brain was found to be typically around 20:1. With this ratio, subvoxel shifts are detectable at steep signal intensity boundaries such as characteristically occur between cerebral tissue and cerebrospinal fluid. If serial images are aligned very accurately to a very small part of a voxel (in order to keep misregistration errors below the image noise level) then small changes in signal intensity and subvoxel shifts of the order of a few percent of a voxel produced by genuine changes in the position, shape or size of the brain may be detectable as a result of the signal changes produced on accurately registered difference images.

From the foregoing, it is clearly of the utmost importance to use methods that produce very accurate registration of serially acquired images. Let us suppose that the second, follow-up, image is a linear translation in one direction such that the image is displaced from the first in the row, column or slice direction of the image matrix by nd , where n is an integer and d is the voxel dimension in the direction of displacement. Then registration would

simply consist of replacing the value of the j th voxel by that of the $(j+n)$ th voxel. A precise correction of the linear translation would result. This procedure can be carried out in all three linear mutually orthogonal dimensions. Difficulties arise, however, if n is non-integer, and/or a rotation or rotations is/are required, since shifts of a fraction of a voxel are then required. In both cases voxel values must be interpolated from neighbouring voxel values, that is, image interpolation is required.

Fractional voxel shifts without distortion require the use of an interpolation function appropriate to the nature of the MR data. As images are acquired in the Fourier domain with data obtained over a bounded region of k -space, their frequency content is strictly band-limited. The in-plane point spread function for MR images is therefore the sinc function (Jain, 1989), where $\text{sinc } z = (\sin z)/z$. In this expression, z can be any complex number. (The domain of this function is not the complex plane punctuated at zero; z can take the value zero without producing a singularity, with $\text{sinc } 0$ equal to one.) Therefore it should be possible to use sinc interpolation to derive interstitial data values without introducing errors. Of course true three-dimensional acquisitions, employing phase encoding in the slice direction as well as in plane, are band-limited in all directions and can therefore be interpolated correctly using this function (Hajnal et al., 1995). (Note that Fourier interpolation was not chosen owing to the increased computer storage space needed. For instance, a shift of one-hundredth of a voxel in any direction would require enlargement of a three-dimensional data set by a factor of a million.)

Representing the intensities of a three-dimensional voxel matrix corresponding to an image data set by $\mathbf{P}(X, Y, Z)$, where the orthogonal Cartesian coordinates X, Y, Z are integers, we wish to interpolate the intensity $\mathbf{P}(x, y, z)$, where x, y, z are real but not necessarily integers. For band-limited MR images, this can be achieved without error by setting $\mathbf{P}(x, y, z)$ equal to $\sum_X \sum_Y \sum_Z \mathbf{P}(X, Y, Z) \text{sinc}[\pi(x-X)] \text{sinc}[\pi(y-Y)] \text{sinc}[\pi(z-Z)]$. However, this is clearly computationally demanding. Taking into account also that, as the modulus of z increases the value of $\text{sinc } z$ tends to decrease, ignoring the oscillatory nature of the function for large values of $\text{mod } z$, so that, in general, the more distant a voxel the smaller its contribution to the sinc interpolation, it is reasonable to truncate the interpolation by limiting the calculation to a cubic volume containing $(2R+2)^3$ voxels centred on the target point for which an intensity is to be calculated. With R set to zero only the nearest neighbours are included in the interpolation. Furthermore, to avoid truncation errors derived from the oscillating nature of the sinc function, each of the three sinc functions

in the above equation is multiplied by an apodizing Hanning window function of the form $H(\alpha; R) = 1/2[1 + \cos(\pi\alpha/(R+1))]$, where $\alpha = x - X$, $y - Y$, $z - Z$.

For matching MR images to each other, Hajnal et al. (1995) chose to use a least squares method as it has the advantages of conceptual clarity, ease of interpretation, and ready availability of appropriate optimization algorithms. It also provides a direct estimate of the statistical uncertainty of the positional match achieved. The subvoxel registration computer program uses the Levenburg-Marquardt algorithm to minimize a chi-squared function of goodness-of-fit (Puri, 2004a). After a minimum value of chi-squared is found, the three translations and three rotation angles required for full three-dimensional registration are stored. If A and B represent the MR images that are to be positionally matched, then the registration coordinates are used to re-slice B to match A using sinc interpolation. A larger value of R is generally used for re-slicing than that used for the determination of the registration coordinates in order to minimize intensity errors in the final images.

The precision of the positional match achieved depends on the competition between structure and noise in the images, and also on genuine differences existing between the objects depicted in the images. The first factor can be characterized as the statistical uncertainty of the positional match, while the second factor may cause systematic registration changes. Both of these are now briefly described.

Consider the case in which A and B only differ with respect to their noise content. Then the minimum value of chi-squared will be $\chi^2 = \sum_{\text{voxels}} [s_A^2 + s_B^2]$, where s_A is the standard deviation of the noise content of image A, and s_B the standard deviation of the noise content of image B. (It is assumed that s_A^2 and s_B^2 have a correlation of zero.) Once an optimal value of chi-squared has been found, the subvoxel registration computer program then routinely calculates an unbiased estimation of the standard deviations of the registration coordinates using the inverse of the Hessian matrix of second derivatives of χ^2 . If, however, the minimum value of chi-squared found by the program is greater than the right-hand side of the last equation, then this implies either that the program has failed to find the global minimum value, or that there exist structural differences between A and B that are not associated with their noise content.

Genuine structural differences between the objects depicted in images A and B lead to a systematic effect on the subvoxel registration coordinates; the optimization procedure leads to a positional match that balances these differences against differences induced by positional shifts (Hajnal & Bydder, 1997).

When looking for genuine structural cerebral changes, it is important to exclude variable deformation of soft tissues in the head and neck outside of the skull. This is achieved by using image segmentation. For neuroimaging this involves removing the skull, scalp, facial tissues and neck tissues, so that only the brain and immediately adjacent tissues and fluids are used for registration. This is achieved by replicating the A image set and then editing it using an automated segmentation algorithm (Marshall et al., 1990; Saeed, 1990) that leaves only the brain and its immediate surrounds, with all other voxels set to zero. A non-zero threshold in the registration program then restricts the calculation to relevant voxels.

To test the results of subvoxel registration, difference images are produced by subtracting the registered images. For serially acquired neuroimages in which no anatomical changes in the brain have occurred, precise positional matching and accurate interpolation ensures that the tissues signals are reduced to the background noise level after image subtraction (Bydder & Hajnal, 1997). Any residual signals detected on registered subtraction images can be ascribed to irreconcilable image differences resulting from real structural changes.

A number of phantom studies have been carried out by Bydder and Hajnal (1997) that validate these techniques. Typical alignments in linear dimensions of less than 0.01 mm were reached, and residual signals caused by misregistration were reduced to the noise floor. Serial scans of the brain in normal volunteers have also validated these techniques. In a typical example quoted by Bydder and Hajnal (1997), in which the same adult male volunteer was scanned twice on the same day using true 3D rf-spoiled T_1 -weighted acquisitions, the mid-sagittal difference image (following image segmentation, subvoxel registration, and image subtraction) showed that almost all the structures within the brain had been reduced to below noise level, with just one exception: the straight sinus could be visualized, probably indicating a change in size between the scans.

Semi-automated quantification of ventricular volumes

This method was devised by Nadeem Saeed and Basant Puri during the late 1990s. It requires three steps: the drawing of contours; threshold computation and the creation of binary images; and the extraction of the ventricular components and their measurement.

Contours are drawn that fully enclose the ventricles. This operation is performed on each

transverse slice and it approximately defines an area in which the ventricles can be seen. These contours are drawn on the basis of neuroanatomical knowledge, taking care to avoid, in particular, the anterior and posterior parts of the corpus callosum in the relevant transverse brain slices. This operation is performed with the aid of a mouse linked to the image display on the workstation. To speed up the contouring process, it is possible sometimes to replicate contours from neighbouring slices.

The next stage entails the installation of circular regions of interest (ROI) of radius 3 mm to lie fully within the lateral ventricles. The threshold, T_v , needed to isolate the ventricles uses the following formula devised by me:

$$T_v = \frac{2}{n} \sum_{i=1}^n M_i + s \quad (1)$$

where: M_i = the intensity in the i th ROI; n = the number of ROIs; and s = the standard deviation of the intensities of the n ROIs.

The image is then thresholded to create a binary image consisting of 'black' and 'white' voxels only (Gonzalez & Woods, 1992).

A contour-following algorithm (Herman & Liu, 1978) is implemented to operate on all binary components obtained from the previous step. The contour-following algorithm extracts the boundary which is then coded using the Freeman 8-way chain code which provides dimensional and positional measurements of the isolated structures. The latter measurement is monitored to ignore all isolated structures that lie outside the operator-drawn contour. This means that the centre of mass of each isolated structure has to lie within the operator-drawn contour in order to qualify as belonging to the ventricular system. The dimensional calculation is used to determine the volume of the ventricular system in a particular transverse slice. All the ventricular components can be extracted automatically within the manually drawn contour using the contour-following procedure. In addition, it has been found to be useful to enable all voxels within the extracted contours to be filled by 'white' voxels when the choroid plexi are to be included in the computation of the ventricular volume. (The choroid plexus has a high signal intensity compared with cerebrospinal fluid on T_1 -weighted images and so may not be isolated by the threshold operation.) If this contour fill option is selected then the extracted contour is completely filled with 'white' voxels; otherwise it is filled with only the 'white' voxels that appear in the thresholded image.

The 'white' voxel count in all the extracted contours corresponding to all the slices are then

summed, to give the ventricular volume in voxels (including fractional voxels). Finally, the product of this sum and the volume of a single voxel expressed in cubic millimetres gives the volume of the segmented ventricles in cubic millimetres.

In phantom studies of a model of the brain, this technique has been found to give a 'ventricular' (phantom fluid-compartment) volume that differs by less than 1% from the calculated volume (according to the mass, density and temperature of the fluid compartment), while serial scanning with the phantom repositioned in the scanner led to a difference in 'ventricular' volume between two scans of just 0.0046% (Saeed, Puri, Oatridge, Hajnal, & Young, 1998).

Applications to neurological and psychiatric disorders following fatty acid intervention

The first patient with schizophrenia to be treated solely with the n-3 long-chain polyunsaturated fatty acid eicosapentaenoic acid (EPA) showed a remarkable and sustained remission of positive and negative symptoms starting within a month of this intervention (Puri & Richardson, 1998). His brain was scanned with high-resolution MRI both at one year and six months before starting the EPA, while he remained antipsychotic drug-free. He underwent MRI brain scanning again at baseline (on starting the EPA), and then after taking the fatty acid daily for six months. Using the two techniques described in the previous section, it was found that cerebral atrophy had been taking place during the year before taking the EPA, with an increase in lateral ventricular volume occurring from 18,750 mm³ (at minus one year with respect to EPA treatment), through 19,440 mm³ (at minus six months) to 19,800 mm³ at baseline. Normalizing these volumes to the total brain volume at each scan gave corresponding values of the ventricle-to-brain ratios (VBRs) of 0.0147, 0.0153 and 0.0155 (Puri et al., 2000; Puri & Richardson, 2003). After six months of taking EPA (and no other medication), there was a reversal of this cerebral atrophy, with the lateral ventricular volume being 18,850 mm³ and the corresponding VBR falling back to 0.0148. These results were the first to indicate that EPA might be able to reverse cerebral atrophy, at least in schizophrenia, as well as to treat positive and negative symptoms in this disorder.

The first published case of a patient with depression to be treated with EPA (Puri et al., 2002a) underwent high-resolution MRI brain scanning at baseline and after nine months of taking the fatty acid. This was a severe case of treatment-resistant depression in a 21-year-old male student with a seven-year history of unremitting

depressive symptoms. After the addition of ultra-pure EPA (in the form of the ethyl ester) there was rapid clinical improvement, including the cessation within one month of previously unremitting severe suicidal ideation; there was also a marked improvement in social phobia symptomatology. There was progressive clinical improvement, and by nine months the patient's depressive symptoms had disappeared altogether, without any evident adverse effects from the EPA. Analysis of the subvoxel-registered MRI subtraction images showed that, during the nine-month period of treatment with EPA, complex changes had taken place in the patient's brain including changes in the cerebral cortex and ventricular system. Overall, a reduction occurred in the volume of the lateral ventricles (Puri, Counsell, Hamilton, Richardson, & Horrobin, 2001; Puri, 2003a).

There is evidence of an association between chronic fatigue syndrome (or myalgic encephalomyelitis, M.E.), a disorder of currently unknown aetiology, and fatty acids (Puri, 2004b). Indeed, sole treatment with a combination of ultra-pure EPA and a mixture of n-6 long-chain polyunsaturated fatty acids, as found in particular in the VegEPA supplement, has been found to offer therapeutic advantages, particularly when used in combination with certain cofactors (Puri, 2005). In the first neuro-imaging study of fatty acid treatment in a patient with chronic fatigue syndrome, high-resolution MRI brain scanning was carried out at baseline and after 16 weeks. The female patient, who was in her mid-twenties, had a six-year history of unremitting symptoms of chronic fatigue syndrome. Following 16 weeks' treatment with the fatty acids there was a marked clinical improvement (Puri et al., 2004). Application of the techniques described in the previous section showed that during this period of fatty acid intervention there was a reduction in the lateral ventricular volume, from 28,940 mm³ to 23,660 mm³.

Following the discovery by Professor Krishna Vaddadi in the 1990s that fatty acids could beneficially modify the clinical course of Huntington's disease (see Vaddadi, 2003), a six-month randomized, placebo-controlled pilot study of EPA was carried out in seven inpatients with advanced (clinical stage III) Huntington's disease. Three of the patients were randomized to the EPA group and the remaining four to the placebo group. Using subvoxel registration of the six-month follow-up MRI brain scans with the baseline scans, the subtraction images showed that while the placebo was associated with progressive cerebral atrophy, as would be expected in this disorder, treatment with EPA was associated with a reverse process (Puri et al., 2002b; Puri, 2003b).

Discussion and conclusions

This paper has shown how the two recently developed image analysis techniques described can be successfully applied to the study of structural changes in the adult brain associated with fatty acid intervention. In particular, it would appear that EPA may be associated with clinical benefits in disorders as diverse as schizophrenia, depression, chronic fatigue syndrome and Huntington's disease, and that this benefit may be measurable by MRI in future rigorous clinical trials.

References

- Bydder, G. M., & Hajnal, J. V. (1997). Registration and subtraction of serial MRI—Part 2: Image interpretation. In W. G. Bradley & G. M. Bydder (Eds.), *Advanced MR Imaging Techniques*. London: Martin Dunitz Freeman.
- Gonzalez, R. C., & Woods, R. E. (1992). *Digital Image Processing*. New York: Addison-Wesley.
- Hajnal, J. V., Saeed, N., Soar, E. J., Oatridge, A., Young, I. R., & Bydder, G. M. (1995). A registration and interpolation procedure for subvoxel matching of serially acquired MR images. *Journal of Computer Assisted Tomography*, 19, 289–296.
- Hajnal, J. V., & Bydder, G. M. (1997). Registration and subtraction of serial MRI—Part 1: Technique. In W. G. Bradley & G. M. Bydder (Eds.), *Advanced MR Imaging Techniques*. London: Martin Dunitz Freeman.
- Herman, G. T., & Liu, H. K. (1978). Dynamic boundary surface detection. *Computer Graphics Image Processing*, 7, 130–138.
- Jain, A. K. (1989). *Fundamentals of Digital Image Processing*. Englewood Cliffs: Prentice Hall.
- Marshall, S., Saeed, N., Young, I. H., Durrani, T. S., Dalton, B., & DuBoulay, G. (1990). System for automatic patient realignment of the head in magnetic resonance imaging. *IEEE Proceedings, Artificial Intelligence in Signal Processing*, 137, 319–330.
- Puri, B. K. (2003a). A possible role for fatty acid treatment in paediatric depression. In M. Peet, I. Glen & D. F. Horrobin (Eds.), *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (pp. 543–548). Carnforth: Marius Press.
- Puri, B. K. (2003b). A randomised trial of ethyl eicosapentaenoate in patients with end-stage Huntington's disease. In M. Peet, I. Glen & D. F. Horrobin (Eds.), *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (pp. 575–579). Carnforth: Marius Press.
- Puri, B. K. (2004a). Monomodal rigid-body registration and applications to the investigation of the effects of eicosapentaenoic acid intervention in neuropsychiatric disorders. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 71, 177–179.
- Puri, B. K. (2004b). The use of eicosapentaenoic acid in the treatment of chronic fatigue syndrome. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 70, 399–401.
- Puri, B. K. (2005). *Chronic fatigue syndrome*. London: Hammersmith Press.
- Puri, B. K., & Richardson, A. J. (1998). Sustained remission of positive and negative symptoms of schizophrenia following treatment with eicosapentaenoic acid. *Archives of General Psychiatry*, 55, 188–189.

- Puri, B. K., & Richardson, A. J. (2003). Long-term follow-up of a single patient with schizophrenia treated with ethyl-EPA alone. In M. Peet, I. Glen & D. F. Horrobin (Eds.), *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (pp. 377–389). Carnforth: Marius Press.
- Puri, B. K., Bydder, G. M., Counsell, S. J., et al. (2002b). MRI and neuropsychological improvement in Huntington disease following ethyl-EPA treatment. *NeuroReport*, *13*, 123–126.
- Puri, B. K., Counsell, S. J., Hamilton, G., Richardson, A. J., & Horrobin, D. F. (2001). Eicosapentaenoic acid treatment in treatment-resistant depression associated with symptom remission, structural brain changes and reduced neuronal phospholipid turnover. *International Journal of Clinical Practice*, *55*, 560–563.
- Puri, B. K., Counsell, S. J., Richardson, A. J., & Horrobin, D. F. (2002a). Eicosapentaenoic acid in treatment-resistant depression. *Archives of General Psychiatry*, *59*, 91–92.
- Puri, B. K., Holmes, J., & Hamilton, G. (2004). Eicosapentaenoic acid-rich essential fatty acid supplementation in chronic fatigue syndrome associated with symptom remission and structural brain changes. *International Journal of Clinical Practice*, *58*, 297–299.
- Puri, B. K., Richardson, A. J., Horrobin, D. F., et al. (2000). Eicosapentaenoic acid treatment in schizophrenia associated with symptom remission, normalisation of blood fatty acids, reduced neuronal membrane phospholipid turnover and structural brain changes. *International Journal of Clinical Practice*, *54*, 57–63.
- Saeed, N. (1990). Application of digital image processing in magnetic resonance scanning. PhD thesis, Signal Processing Division. Glasgow: University of Strathclyde.
- Saeed, N., Puri, B. K., Oatridge, A., Hajnal, J. V., & Young, I. R. (1998). Two methods for semi-automated quantification of changes in ventricular volume and their use in schizophrenia. *Magnetic Resonance Imaging*, *16*, 1237–1247.
- Vaddadi, K. S. (2003). Essential fatty acids in the treatment of Huntington's disease. In M. Peet, I. Glen & D. F. Horrobin (Eds.), *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (pp. 565–574). Carnforth: Marius Press.