Magnetic Resonance Spectroscopy Detects Biochemical Changes in the Brain Associated with Chronic Low Back Pain: A Preliminary Report

Philip J. Siddall, MBBS, PhD,* Peter Stanwell, BSc,† Annie Woodhouse, PhD,* Ray L. Somorjai, PhD,‡ Brion Dolenko, MSc,‡ Alexander Nikulin, PhD,‡ Roger Bourne, PhD,† Uwe Himmelreich, PhD,† Cynthia Lean, PhD,† Michael J. Cousins, AM, MD,* and Carolyn E. Mountford, DPhil†

*Pain Management Research Institute, University of Sydney, Royal North Shore Hospital and †Institute for Magnetic Resonance Research and Department of Magnetic Resonance in Medicine, University of Sydney, Sydney, Australia; and ‡Institute for Biodiagnostics, National Research Council Canada, Winnipeg, Canada

Magnetic resonance (MR) spectroscopy is a noninvasive technique that can be used to detect and measure the concentration of metabolites and neurotransmitters in the brain and other organs. We used *in vivo* ^{1}H MR spectroscopy in subjects with low back pain compared with control subjects to detect alterations in biochemistry in three brain regions associated with pain processing. A pattern recognition approach was used to determine whether it was possible to discriminate accurately subjects with low back pain from control subjects based on MR spectroscopy. MR spectra were obtained from the prefrontal cortex, anterior cingulate cortex, and thalamus of 32 subjects with low back pain and 33 control subjects without pain. Spectra were analyzed and compared between groups using a pattern recognition method (Statistical Classification Strategy). Using this approach, it was possible to discriminate between subjects with low back pain and control subjects with accuracies of 100%, 99%, and 97% using spectra obtained from the anterior cingulate cortex, thalamus, and prefrontal cortex, respectively. These results demonstrate that MR spectroscopy, in combination with an appropriate pattern recognition approach, is able to detect brain biochemical changes associated with chronic pain with a high degree of accuracy.

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The assessment of chronic low back pain, one of the most prevalent and disabling conditions in medical practice (1–3), is hampered by the lack of a rigorous objective method that can reliably detect and measure biochemical or physiological changes associated with the pain (4). Although magnetic resonance imaging (MRI) of the spine can often identify pathological changes contributing to back pain, there is a poor correlation between the pathological abnormalities and the pain report (4,5). This highlights the subjective nature of pain diagnosis and the interplay of pathology, nociceptive inputs, psychological processes, and pain behavior (6–8). If a method could be developed to provide an objective, accurate indicator of the presence of pain, it would radically change both the diagnosis and treatment of persistent pain.

Magnetic resonance (MR) spectroscopy can monitor biochemical changes in specific regions of the brain associated with disease processes (9,10). In contrast to standard MRI, which can produce detailed images of the brain, or functional MRI, which characterizes temporal differences in brain activity in response to stimulation, MR spectroscopy characterizes the levels of metabolites present within the brain region of interest.

A number of studies indicate that MR spectroscopy can detect biochemical changes associated with functional brain abnormalities, such as epilepsy and dyslexia (11,12) and, more recently, pain (13–15). Low back pain (13), complex regional pain syndrome (14), and neuropathic spinal cord injury pain (15) have been associated with biochemical changes in the thalamus

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Address correspondence and reprint requests to Dr. Philip Siddall, Pain Management Research Institute, Royal North Shore Hospital, St. Leonards NSW 2065, Australia. Address e-mail to phils@med.usyd.edu.au

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and frontal cortex. However, the relatively few subjects in each cohort ($N \leq 12$), and confounding factors, such as mood (16), made it difficult to identify specific biochemical markers of persistent pain.

We have demonstrated that it is possible to provide medical diagnosis and prognosis with accuracies of more than 95% using MR spectroscopy in conjunction with a particular pattern recognition method developed specifically for biomedical spectroscopy data (Statistical Classification Strategy [SCS]) (17–20). The advantage of this method is that it can detect small differences in spectral patterns that are not evident upon visual inspection of spectra. For clinicians, it offers the possibility to objectively discriminate between those people with pain and those without pain and has the potential to suggest alterations in specific metabolites associated with each type of pain. This may in the future permit the development of "brain biochemical fingerprints" of different pain states. Such fingerprints will serve as a basis for selecting treatments.

Therefore, the aim of this study was to determine whether MR spectroscopy of certain brain regions, combined with an appropriate pattern recognition method, could discriminate subjects with low back pain from control subjects without pain based on alterations in regional brain biochemistry.

Methods

After study approval by the institutional Human Research Ethics Committee, subjects with low back pain were recruited through a multidisciplinary pain management center in a large teaching hospital. Thirty-two subjects with low back pain volunteered to take part in the study. Thirty-three age- and sex-matched volunteer control subjects without pain were also recruited. Written informed consent was obtained from all subjects before taking part in the study. All subjects with pain underwent a comprehensive multidisciplinary evaluation by a pain physician, physiotherapist, and psychiatrist or clinical psychologist. Evaluation included history, examination, standardized instruments for assessment of pain (Short Form McGill Pain Questionnaire), mood (Depression Anxiety Stress Scales), disability (Roland & Morris Disability Questionnaire), other measures of psychological function (Pain Self-Efficacy Questionnaire), and appropriate imaging (radiographs, computerized tomography and/or MRI of the lumbosacral spine). The specific physical diagnosis, based on presumed causative factors or pathology, varied among subjects in the low back pain group. However, subjects were assessed to determine whether their low back pain was predominantly nociceptive or neuropathic in origin.

A diagnosis of nociceptive pain was supported by descriptors, such as dull and aching, tenderness to palpation of deep structures, pain with movement or activity and radiological evidence of pathology involving musculoskeletal structures consistent with findings on examination. A diagnosis of neuropathic pain was supported by descriptors such as electric, shock-like, and burning, the presence of tingling and numbness, tenderness of superficial structures in regions close to a region of sensory deficit, and radiological evidence of pathology in neural structures consistent with findings on examination. Given the lack of a definitive diagnostic technique in the assessment of low back pain, subjects with features suggestive of both types of pain were included. Therefore, the "diagnosis" of low back pain included subjects with pain arising from this region, irrespective of cause. However, subjects were only included if, after multidisciplinary assessment, they were determined as having significant nociceptive or neuropathic contributors to their experience of pain and met the criteria for chronic pain as defined by the International Association for the Study of Pain (21).

Pain had to be present for at least 6 months and be rated as moderate or severe. Medication use was recorded; however, for ethical reasons (acceptability to subjects, as well as the risk of possible medical complications associated with ceasing prescribed medications), medication usage was not altered before MR spectroscopy.

MR Spectroscopy

MR examinations were performed in a 1.5 T clinical MRI scanner (General Electric, Milwaukee, WI), using the manufacturer's standard transmit/receive quadrature head coil. Single voxel (volume 8 cm^3) MR spectra were obtained from three anatomical regions of interest (left anterior cingulate cortex, left prefrontal cortex, left thalamus) (Fig. 1). These regions were chosen on the basis of their known involvement in different aspects of nociceptive processing (22).

MR spectra were acquired using the stimulated-echo acquisition mode pulse sequence (TE, 25 ms; TR, 1500 ms), 256 signal averages were acquired per data frame with eight-step phase cycling (2048 data points per spectroscopy frame with a spectral bandwidth of 2500 Hz), resulting in an acquisition time of approximately 6.5 min. Magnetic field homogeneity was optimized for the selected spectroscopy volume of interest by manual shimming. The resulting peak width of water at half-maximum was 16 Hz or better for all voxels. The chemical-shift-selective suppression pulse sequence (23) was used for water suppression. The amplitude of the third chemical-shift-selective pulse was manually adjusted to achieve optimum water suppression before signal acquisition. MR spectra were shift-calibrated to the *N*-acetyl aspartate resonance at 2.01 ppm. Normalized magnitude spectra

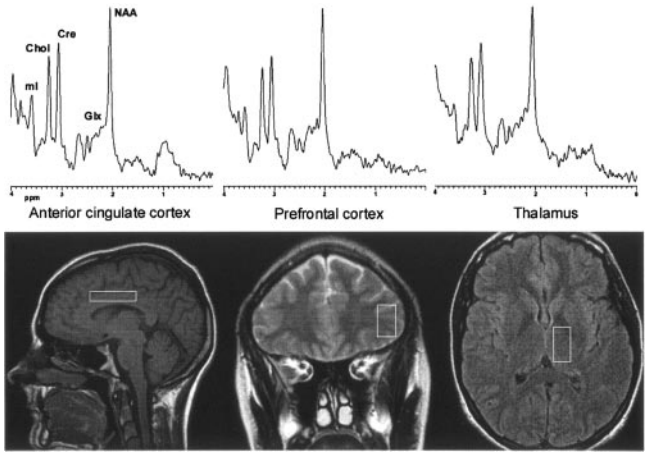

Figure 1. (*A) Top row,* Typical single-voxel magnetic resonance (MR) spectra collected from the anterior cingulate cortex, prefrontal cortex, and thalamus of a low back pain subject using stimulated-echo acquisition mode prescription (TE, 25 ms; TR, 1500 ms; 256 acquistions). (*B) Bottom row,* box indicates the placement of each single-voxel measurement on the MR image. Chol = choline; Cre = creatine; Glx = glutamine/glutamate; mI = myoinositol; NAA = *N*-acetyl aspartate.

from each raw data set were summed to create average cohort spectra for visual comparison.

Spectral Analysis

Spectra were analyzed in two ways: by comparison of individual resonances and assignment of resonances from the literature (24) and by the SCS, a designed pattern recognition method specifically developed for biomedical spectroscopy data (17,25). Data outside the 0–4 ppm region of each magnitude spectrum were excluded from analysis to reduce the effect of spectral artifacts arising from the implementation of water suppression. First derivatives of normalized spectra, and rank-ordered versions of these (where each value is replaced by an integer representing its rank), were used as input to the feature selection/extraction stage of the SCS-based approach. Feature selection was performed by the Optimum Region Selection method (26). Optimum Region Selection uses a genetic algorithm and leave-one-out cross-validated Linear Discriminant Analysis (LDA) to select a small number (5–6) of maximally discriminatory spectral regions.

The signal averages in the Optimum Region Selection-determined subregions were then used to create LDA-based classifiers. These classifiers are developed by a cross-validated bootstrap-inspired method (17,27). Half the spectra from each class are randomly selected (the training set) and used to train an LDA classifier. The resulting classifiers are used to classify the remaining spectra (the test set) not included in the training set. The process is repeated up to 10,000 times. The average LDA coefficients, weighted according to the accuracy of classification of the test sets, are used as the final LDA classifier. The bootstrapping method reduces the possibility of "overfitting" the data, because the final classification coefficients are derived only from independently validated classifiers. For the thalamus and prefrontal cortex data, the ultimate classifier was created by combining the outcome probabilities of the two LDA classifiers constructed using first derivative and rank-ordered first derivative data. The combining method was Wolpert's Stacked Generalizer (28), which uses the output class probabilities obtained by the individual LDA classifiers as input features for the combined classifier. For the anterior cingulate cortex, 100% classification accuracy was achieved with a single LDA classifier (derived from rank-ordered first derivative spectra) alone, and a combined classifier was not required for this brain region. The outputs of the classifiers (single LDA or generalized combination) are class assignment probabilities. Samples are assigned to the class (pain/no pain) for which the assignment probability is higher.

Results

Typical spectra from each brain region (prefrontal cortex, anterior cingulate cortex, and thalamus) of a subject with low back pain are shown in Figure 1. Resonance assignments are as reported in Danielson and Ross (10).

When the SCS-based pattern recognition method was applied to the MR spectra, it was able to discriminate low back-pain subjects from control subjects with an accuracy of 100% from the anterior cingulate cortex spectra, 98.5% from the thalamus spectra, and 96.6% from the prefrontal cortex spectra. The spectral regions identified by the Optimum Region Selection algorithm as optimal for discrimination between the pain and no pain groups are listed in Table 1. Because the searches were nonexhaustive, and the sample sizes small, there may be other spectral regions, not identified here, that are comparable in discriminatory power (29). Thus it is not possible from the data analyzed to unequivocally identify the metabolites associated with the detected spectral patterns characterizing the pain and no-pain groups.

Discussion

The ability of MR spectroscopy to discriminate subjects with persistent low back pain from control subjects with accuracies of 97%–100% based on regional brain biochemistry is a major step toward having an objective diagnostic technique in the assessment of persistent pain. Furthermore, the combination of MR spectroscopy with the appropriate pattern recognition method (in this case, SCS) can provide information on

Table 1. Discriminative Ability of Statistical Classification Strategy with Analysis of Each Brain Region

Brain region	Sensitivity	Specificity	Accuracy	Spectral regions (ppm)
Prefrontal cortex	96.7% (A + B)	96.6% (A + B)	96.6% (A + B)	A. 3.10–3.16, 2.71–2.79, 2.26–2.34, 1.40–1.48, 1.27–1.28, 0.56–0.65 B. 3.61–3.66, 3.10–3.16, 2.12–2.19, 1.36–1.50, 0.88–0.96, 0.53–0.65
Anterior cingulate cortex	100%	100%	100%	B. 3.44–3.50, 2.77–2.83, 2.32–2.39, 1.42–1.47, 0.50–0.55
Thalamus	96.9% (A + B)	100% (A + B)	98.5% (A + B)	A. 3.22–3.25, 3.12–3.16, 2.35–2.39, 2.15–2.27, 1.86–1.88, 0.66–0.74 B. 3.32–3.39, 3.21–3.26, 3.00–3.05, 1.55–1.64, 0.75–0.85, 0.65–0.67

A indicates first derivatives, and B indicates rank-ordered first derivatives. ppm = parts per million.

the biochemical profile specific to each brain region when pain is present or absent.

The SCS, using MR spectra, has been successfully applied to other medical situations. It is important to note that SCS-based analyses have been reported in numerous publications and have been demonstrated to be highly reliable for early diagnosis of breast and prostate cancer (19,20). Another example is the analysis of biopsies taken from primary malignant breast lesions. Using only MR spectra from the primary tumor, the SCS-based analysis was successful in identifying patients whose cancer spread to the nodes (30). This program is now undergoing clinical acceptance testing.

Detection of alterations in brain biochemistry occurring in persistent pain states presents two opportunities: first, the ability to detect changes associated with specific pain conditions (a fingerprint) leading to a better understanding of underlying mechanisms and potential therapies and assisting in pain diagnosis and, second, the ability to detect changes specific to individual patients. The latter opportunity offers the possibility of tailoring treatment on an individual basis.

This is not the first study to use MR spectroscopy to identify regional brain biochemical changes associated with chronic pain. Initial studies using this technique identified alterations in the concentrations of transmitters (such as *N*-acetyl aspartate) in such regions as the dorsolateral prefrontal cortex in subjects with low back pain (13). Several other studies have also reported alterations in brain biochemistry in association with complex regional pain syndrome (14,31) and neuropathic pain after spinal cord injury (15). It has been suggested that such changes may be markers of chronic pain and provided hope that MR spectroscopy might provide a noninvasive technique for the detection of objective markers associated with the presence of chronic pain.

One of the limitations of MR spectroscopy is that it detects biochemical changes in the brain that may be due to other factors not directly associated with pain. For example, biochemical changes may occur in the brain with mood dysfunction or medication use. A number of studies demonstrate alterations in regional brain chemistry in altered mood states such as depression (32) and anxiety (33). In fact, although Grachev et al. (13) initially suggested that decreases in prefrontal *N*-acetyl aspartate might be indicative of the presence of low back pain, a subsequent study demonstrated that there was a stronger association of reduced *N*-acetyl aspartate with depression than with pain (16).

Medication use may also alter brain biochemistry and therefore make it difficult to detect changes specifically associated with the presence of pain. Although it would be ideal to scan subjects who are not using medications, in practice this is extremely difficult for several reasons. First, subjects in severe pain are extremely resistant to withdrawal of analgesia and there are ethical issues if subjects are left without analgesia for the period of time required for drug washout; second, many low back pain and control subjects are taking other medications that also may affect brain biochemistry, and cessation of all medications may cause serious medical complications; and third, a reduction in medications may, in itself, alter brain biochemistry and thus cause the very situation that we are trying to avoid.

To overcome some of these difficulties, we have used a different method of spectral analysis to determine whether it is possible to distinguish subjects with low back pain from subjects without pain. This study differs from previous reports on the use of MR spectroscopy in that it examines a larger number of subjects and applies the SCS-based analysis to determine whether it is possible to distinguish the pain cohort from volunteers.

Previous studies have illustrated the difficulty of the use of visual inspection of the data, collected at 1.5 T, to assess conditions as complex as persistent pain. In addition, a major advantage of the SCS is that it identifies those spectral regions and hence the chemicals that were used to make the distinction between the categories. This will permit further studies that focus on identifying and measuring specific chemical changes within these brain regions. For example, the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter γ-aminobutyric acid are both contained within the spectral region of interest and are intimately involved in sensory processing. Identifying alterations in concentrations in these transmitters in specific pain populations and even individual patients

will be a potential application. The accurate identification of changes that are specific to persistent pain conditions will, one hopes, lead to the development of new treatments and the ability to match treatments to individual needs.

In conclusion, this study applies the SCS-based analysis to biochemical data obtained from three brain regions using MR spectroscopy. The method distinguishes subjects with low back pain from pain-free volunteers with accuracies of 100%, 99%, and 97% for the anterior cingulate cortex, thalamus, and prefrontal cortex, respectively. These findings promise to provide an objective indicator for the presence of pain as well as to characterize the biochemical changes associated with persistent pain both in pain conditions generally and in individual subjects.

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