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Abnormal brain chemistry in chronic back pain: an in vivo proton magnetic resonance spectroscopy study

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Abstract

The neurobiology of chronic pain, including chronic back pain, is unknown. Structural imaging studies of the spine cannot explain all cases of chronic back pain. Functional brain imaging studies indicate that the brain activation patterns are different between chronic pain patients and normal subjects, and the thalamus, and prefrontal and cingulate cortices are involved in some types of chronic pain. Animal models of chronic pain suggest abnormal spinal cord chemistry. Does chronic pain cause brain chemistry changes? We examined brain chemistry changes in patients with chronic back pain using in vivo single- voxel proton magnetic resonance spectroscopy (^{1}H-MRS). In vivo ^{1}H-MRS was used to measure relative concentrations of N-acetyl aspartate, creatine, choline, glutamate, glutamine, γ-aminobutyric acid, inositol, glucose and lactate in relation to the concentration of creatine. These measurements were performed in six brain regions of nine chronic low back pain patients and 11 normal volunteers. All chronic back pain subjects underwent clinical evaluation and perceptual measures of pain and anxiety. We show that chronic back pain alters the human brain chemistry. Reductions of N-acetyl aspartate and glucose were demonstrated in the dorsolateral prefrontal cortex. Cingulate, sensorimotor, and other brain regions showed no chemical concentration differences. In chronic back pain, the interrelationship between chemicals within and across brain regions was abnormal, and there was a specific relationship between regional chemicals and perceptual measures of pain and anxiety. These findings provide direct evidence of abnormal brain chemistry in chronic back pain, which may be useful in diagnosis and future development of more effective pharmacological treatments.

Keywords: Brain chemistry; Chronic back pain; Dorsolateral prefrontal cortex; Glucose; N-Acetyl aspartate; Proton magnetic resonance spectroscopy

1. Introduction

Back pain afflicts up to 85% of all people at some time in life (Deyo, 1998; Andersson, 1999). In the USA, back pain is the most common cause of activity limitation in people younger than 45 years, the second most frequent reason for visits to the physician, the fifth-ranking cause of admission to hospital, and the third most common cause of surgical procedures (Taylor et al., 1994; Hart et al., 1995). Data from other western countries are similar. When back pain becomes chronic it is highly debilitating and pharmacologically untreatable (Deyo, 1998; Andersson, 1999). Several original reports, editorials and books published during the last 5 years added much to understanding of clinical diagnosis, therapeutic approaches and outcomes for acute and chronic back pain (Deyo, 1994; Jensen et al., 1994; Rossignol, 1995; Frank, 1997; Shekelle, 1999). The diagnostic value of structural neuroimaging studies in back pain, such as a magnetic resonance imaging (MRI) of the lumbar spine, was also discussed (Deyo, 1994; Jensen et al., 1994). The study by Jensen et al. (1994) examined the lumbar spine in people without back pain using MRI and found a high prevalence of lumbar spine abnormalities. Over half the subjects without back pain had bulging disks, and over a quarter had disk protrusions (i.e. herniated disks). Jensen et al. (1994) conclude that the combination of (i) the high prevalence of back pain in the population and (ii) the high prevalence of the lumbar spine abnormalities in people without back pain makes coincidental findings likely. Another MRI study of lumbosacral intervertebral disk abnormalities in pregnant and asymptomatic non-pregnant women raised the same issue, suggesting that structural imaging of the spine cannot predict either the need for surgery or its outcome (Weinreb et al., 1989). Currently,

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the lumbar spine surgery is the main procedure performed after disk abnormalities have been diagnosed. According the US National Hospital Discharge Survey (Taylor et al., 1994), during the 11 years studied (1979–1990), operations among adults for low back pain increased by 55% (from 147 500 in 1979 to 279 000 in 1990). On the other hand, non-surgical admissions to hospitals decreased (from 402 per 100 000 adults in 1979 to 150 per 100 000 adults in 1990). Although it is well known that spine surgery might relieve pain in back patients, most back pain patients improve without a surgical intervention, and psychological factors might be an important predictor for recovery from pain (Waddell et al., 1986; Spengler et al., 1990; Enzmann, 1994). For example, the psychological factor anxiety seems to precede chronic low back pain, whereas depression may develop before or after the onset of back pain (Andersson, 1999).

To date, the neurobiology of chronic pain, including chronic back pain, remains unknown. Studies of visual inputs on phantom pain sensations show that chronic pain has a large cortical component (Ramachandran and Rogers-Ramachandran, 1996). Functional brain imaging studies indicate that the thalamus, and prefrontal and cingulate cortices are involved in some types of chronic pain (Di Piero et al., 1991; Rosen et al., 1994; Hsieh et al., 1995, 1996; Weiller et al., 1995; Peyron et al., 1998; Apkarian et al., 2000), and phantom limb and chronic back pains are causally related with cortical reorganization (Flor et al., 1995, 1997). Studies in animal models of chronic pain indicate changes in peripheral and spinal cord chemistry (Benett, 1994; Stiller et al., 1996; Cui et al., 1997; Besson, 1999; Woolf and Mannion, 1999).

We test the hypothesis that the chronic pain state is associated with abnormal brain chemistry, specifically in the dorsolateral prefrontal cortex (DLPFC), cingulate cortex and the thalamus. These three brain regions were selected based on the results of recent brain imaging studies of chronic pain (Di Piero et al., 1991; Rosen et al., 1994; Hsieh et al., 1995, 1996; Weiller et al., 1995; Peyron et al., 1998; Apkarian et al., 2000). Brain chemistry is compared between nine chronic back pain patients and 11 healthy volunteers (age- and sex-matched). Concentrations of nine metabolites were studied in these three brain regions, using in vivo localized three-dimensional proton magnetic

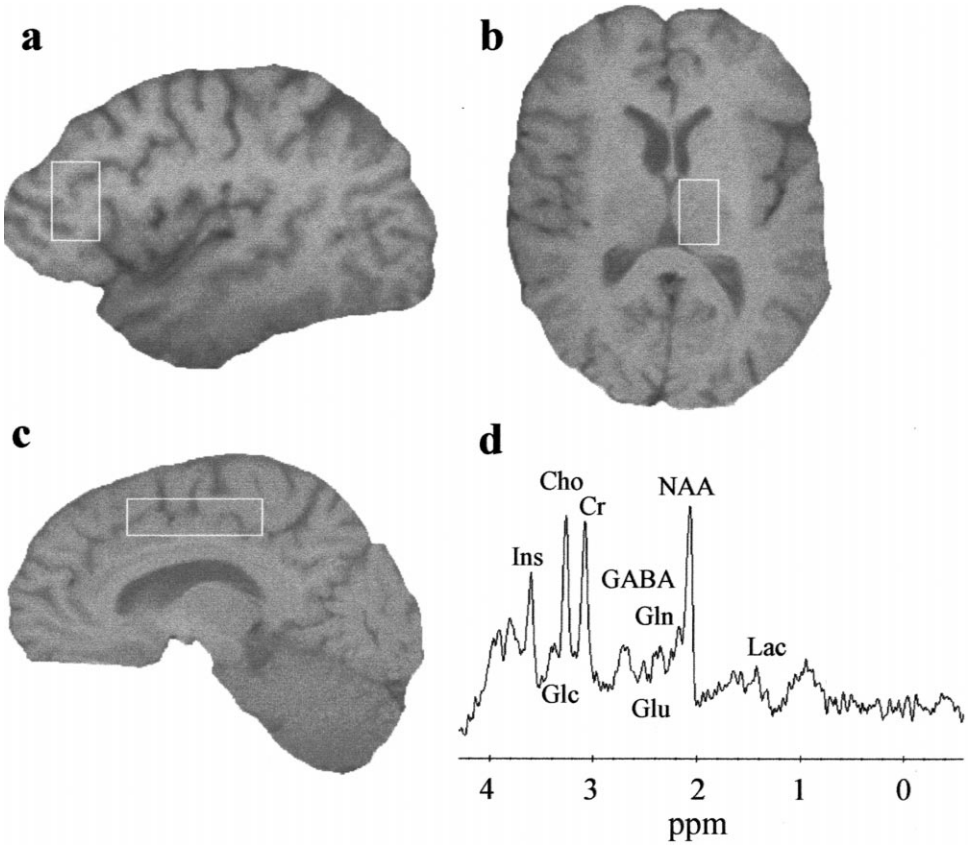

Fig. 1. An in vivo three-dimensional single-voxel proton MRS brain examination. T1-weighted spin echo high-resolution images showing the locations of three voxels in the left hemisphere of a normal subject. (a–c) Voxel sizes and positions of regions of interest for DLPFC, thalamus, and cingulate. (d) A typical in vivo proton MRS spectrum obtained from DLPFC showing localization of three major peaks for N-acetyl aspartate (NAA, 2.02 ppm), creatine/phosphocreatine complex (Cr, 3 ppm), choline (Cho, 3.2 ppm), and smaller peaks for glutamate (Glu, 2.35 ppm), glutamine (Gln, 2.15 ppm), γ-aminobutyric acid (GABA, 2.25 ppm), myo- and scyllo-inositol complex (Ins, 3.60 ppm), glucose (Glc, 3.43 ppm) and lactate (Lac, 1.3 ppm). Chemical shifts are indicated in parts per million (ppm).

resonance spectroscopy (^{1}H-MRS) methodology (Fig. 1). All chronic back pain subjects underwent comprehensive perceptual measures, which included pain (short form of the McGill Pain Questionnaire (SF-MPQ); Melzack, 1987) and anxiety (State–Trait Anxiety Inventory (STAI); Spielberger et al., 1983) questionnaires. We predict that if the brain chemistry is abnormal in chronic pain then these brain chemical changes should be related to the perceptual measures of pain and anxiety. Part of this work was presented at the 9th World Congress on Pain, Vienna in 1999, and the 29th Annual Meeting of Society for Neuroscience, Miami Beach in 1999 (Grachev et al., 1999a,b).

2. Methods

2.1. Subjects

Nine right-handed chronic back pain patients (mean age 45 ± 6 years, range 34–62 years; seven men and two women) and 11 normal volunteers (mean age 44 ± 3 years, range 40–52 years; nine men and two women) participated in the proton MRS study. All normal subjects were recruited by advertisement from the local community. Subjects were matched for age, sex, and handedness (all subjects were right-handed). Initial high resolution MRI of all subjects was obtained and examined by a staff neuroradiologist to exclude brain morphologic abnormality. The lumbar spine in all patients with chronic back pain was examined on MRI scans as part of clinical evaluation. The drugs most commonly used by the studied chronic back pain patients were: (1) non-steroidal anti-inflammatory drugs (six subjects); (2) tricyclic antidepressants (three subjects); (3) opioid analgesics (inconsistently by three subjects); and (4) anti-convulsants (two subjects). In addition, each patient had undergone physical therapy for several years while in chronic pain. All subjects were refrained from medications for at least 24 h before the MRS study. The patients were identified as having chronic back pain based on the criteria of Merskey and Bogduk (1994). All patients had back pain with a duration greater than 1 year (mean duration of pain 9 ± 5 years, range 1–20 years). Each chronic back pain patient completed a perceptual assessment (described below). The general purpose and the procedures were explained to the subjects. All subjects signed a consent form. The Institutional Review Board approved all procedures in this study.

2.2. Localized in vivo 3D single-voxel ^{1}H-MRS brain examination

During brain imaging the subject was placed on the scanner bed, and the whole head gradient coil was positioned over the head. The subject's head was immobilized using a vacuum beanbag. Automated global shimming (part of MRS software package SPECTRO, General Electric) was performed to optimize the magnetic field homogeneity over the entire brain volume, as well as for each specific regional volume.

2.2.1. Acquisition of magnetic resonance images for localization

All MRI and MRS experiments were performed on a 1.5 T General Electric (Signa) clinical imaging instrument. High-resolution sagittal and axial views were used for the selection of volumes of interest. T1-weighted multi-slice spin echo scout images (TR = 500 ms; TE = 12 ms; 2NEX; 256×256 matrix; FOV = 24×24 cm) of the entire brain were obtained with 6.0 mm slice thickness and a 0.5 mm gap between slices, imaging 20 slice locations.

2.2.2. Selection of volume of interest

Localized 3D single-voxel ^{1}H-MRS was then performed in two sagittal (cingulate and DLPFC) and in four axial (thalamus, insula, orbitofrontal and sensorimotor cortex) locations in the left hemisphere of right-handed normal volunteers and back pain patients. Positioning of each 8 cm^3 voxel was performed by an experienced neuroanatomist and adjusted to the individual brain's sulcal topography. We used 8 cm^3 voxels for each analyzed volume: $1.7 \times 3.0 \times 1.6$ cm for DLPFC, $3.0 \times 1.8 \times 1.5$ cm for thalamus, and $5.0 \times 1.6 \times 1.0$ cm for cingulate. Fig. 1 shows these three regional volumes. Other cortical regions that we examined were: insula (voxel size $4.0 \times 2.0 \times 1.0$ cm), sensorimotor cortex ($2.0 \times 1.1 \times 3.5$ cm), orbital frontal cortex ($2.0 \times 2.0 \times 2.0$ cm), and visual cortex ($2.0 \times 2.0 \times 2.0$ cm).

2.2.3. MRS data collection and processing

Proton localized spectra were collected using a simulated-echo acquisition mode (STEAM) sequence (probe-s PSD; TR = 1500 ms; TE = 30 ms). All spectra were transformed into a standardized scale using the Scion Image analysis package (1998, see the web site: http://www.scioncorp.com). Proton spectra were analyzed by measuring heights at specified peaks, with the investigator blinded to both the location and to the subject. The relative concentrations of N-acetyl aspartate (NAA), choline (Cho), glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA), myo- and scyllo-inositol complex (Ins), glucose (Glc) and lactate (Lac) were measured relative to the concentration for creatine/phosphocreatine complex (Cr), which is commonly used as an internal standard. The peak heights ratio method is currently used in clinical ^{1}H-MRS mostly because it is simple and requires no technical expertise or software besides that supplied with the imager. This approach is precise and accurate if the peak height is directly proportional to the peak area. In our study, the width of the specified peaks was almost unchanged across all subjects and brain regions, which was an indication of parallel relationships between the peak heights and the peak areas. Therefore, we used the peak heights ratio method. Fig. 1 shows a typical proton MRS spectrum of the normal human brain

obtained from DLPFC. The MRS spectra are usually characterized by three major peaks: NAA at 2.02 ppm, Cr at 3.0 ppm, and Cho at 3.2 ppm. NAA is the dominant peak in normal adult brain spectra. The Cr spectrum is a combination of creatine and phosphocreatine (Michaelis et al., 1993). This peak was stable across the studied groups and used as an internal standard. The proton Cho signal is a combination of Cho and Cho-containing compounds: Cho plasmogen, glycerophosphorylcholine, phosphorylcholine, cytedine-diphosphate-choline, acetylcholine, and phosphatidylcholine (Michaelis et al., 1993). The other observable metabolites measured in our study were Glu (2.35 ppm), Gln (2.15 ppm), GABA (2.25 ppm), Ins (3.60 ppm), Glc (3.43 ppm) and Lac (1.3 ppm) (Fig. 1). These smaller peaks are contaminated by signals from other metabolites and proteins, although the prominent signal is from the chemicals with which we identify these peaks (Salibi and Brown, 1998).

2.3. Perceptual measures of pain and anxiety

All chronic back pain subjects underwent perceptual measures of pain and anxiety minutes before brain imaging. These tests included assessments of pain (short form of the McGill Pain Questionnaire; Melzack, 1987) and anxiety levels (the State–Trait Anxiety Inventory; Spielberger et al., 1983).

2.3.1. The short-form McGill Pain Questionnaire (SF-MPQ)

The main component of the SF-MPQ consists of 15 descriptors (11 sensory, four affective) which are rated on an intensity scale as follows: 0, none; 1, mild; 2, moderate; 3, severe. The SF-MPQ also includes the Present Pain Intensity (PPI) Index and a visual analogue scale (VAS).

2.3.2. The State–Trait Anxiety Inventory

State anxiety refers to anxiety felt at a particular moment, while trait anxiety refers to a habitual tendency to be anxious over a long period of time. In responding to the STAI State-Anxiety scale (STAI form Y-1), examinees indicate the number that best describes the intensity of their feeling. In responding to the STAI Trait-Anxiety scale (STAI form Y-2), examinees are instructed to indicate how they generally feel by rating the frequency of their feelings of anxiety on a four-point scale. To obtain scores for the S-Anxiety and T-Anxiety scales, the sum of the weighted scores for the 20 items that make up each scale was calculated, taking into account the reversed scores (Spielberger et al., 1983).

2.4. Statistical analyses

Multi-way analysis of variance (ANOVA; STATISTICA, Tulsa, OK) was used to differentiate between back pain patients and volunteers across brain regions and chemicals. The outcome variable was chemical concentration relative to Cr peak. This analysis tests the main hypothesis of the study. Post-hoc comparisons identified specific regional chemical differences. Chemical network differences between patients and volunteers were examined using correlation analysis. Pearson's correlation, calculated for pairs of regional chemicals for each subject grouping, was used as the outcome variable in multi-way ANOVA, where the results are regarded as descriptive metrics. Univariate comparisons were then employed to test specific hypotheses derived from this ANOVA analysis. The latter are interpreted as random factors analysis results. In the back pain patients, the relationship between pain perception and brain regional chemistry was also analyzed using multi-way ANOVA. This analysis was supplemented with a regression analysis where the relationships between regional chemistry and perceptual parameters were explored.

3. Results

The clinical characteristics and results of perceptual measures of pain and anxiety in the nine chronic back pain patients are shown in Table 1. The brain chemistry measurements of the nine patients and 11 normal volunteers we studied are shown in Tables 2 and 3. The original data for chemical concentrations were distributed normally (Kolmogorov–Smirnov $D = 0.07$, $P < 0.01$; $\chi^2 = 54.66$, $P < 10^{-7}$). To quantify differences in the concentration of brain chemicals between chronic back pain patients and healthy volunteers across brain regions we performed a three-way analysis of variance (ANOVA). A 2 (diagnosis) × 3 (brain region) × 9 (chemical) measures ANOVA revealed significant multivariate main effects for diagnosis (effect of chronic back pain) ($F(1,468) = 4.44$, $P < 0.03$), brain region ($F(2,468) = 60.27$, $P < 10^{-7}$), chemical ($F(8,468) = 550.99$, $P < 10^{-7}$), the diagnosis × brain region interaction ($F(2,468) = 4.10$, $P < 0.02$), and for the brain region × chemical interaction ($F(16,468) = 6.80$, $P < 10^{-7}$). Strongest significant multivariate main effects were observed for brain region, chemical, and the brain region × chemical interaction (all $P < 10^{-7}$), which is consistent with our previous report of another group of normal volunteers aged 19–31 years (Grachev and Apkarian, 2000a). The total chemical concentration in patients with chronic back pain was 6.5% lower in DLPFC compared with the same region in healthy volunteers ($F = 12.29$, $P < 0.0005$). The concentration of chemicals in the thalamus and cingulate was not different from those in normal subjects. Regional and chemical specific differences contributed to the total concentration differences between chronic back pain subjects and normal volunteers (Fig. 2). In the DLPFC of chronic back pain subjects, decreased concentration levels were detected for glucose by 17.2% (mean ± SD 0.68 ± 0.11 for normals versus 0.58 ± 0.04 for chronic back pain; $F = 6.05$, $P = 0.01$), N-acetyl aspartate by 7.8% (mean ± SD 1.19 ± 0.04 for

Table 1
Clinical characteristics and perceptual measures for pain and anxiety of nine patients with chronic low back pain

Patient no.	Sex/age (years)	Duration of pain (years)	Lumbar stenosis and herniated disc	Surgery (laminectomy)	Back and leg pain	MPQ-sensory	MPQ-affective	MPQ-total	Visual analogue scale (%)	Present Pain Intensity Index	State anxiety	Trait anxiety
1	M/56	12	Yes	Yes	Yes	21	5	26	59.4	2	46	47
2	M/56	18	Yes	Yes	Yes	15	3	18	40	3	43	44
3	F/50	5	Yes	No	Yes	24	8	32	40.4	1	41	40
4	M/62	20	Yes	Yes	Yes	10	0	10	70.9	2	27	28
5	M/34	1	No	No	Yes	13	7	20	84.8	4	30	31
6	M/46	7	Yes	Yes	Yes	11	0	11	61.3	1	27	34
7	M/42	3	Yes	No	Yes	28	9	37	74	3	51	61
8	M/48	5	Yes	Yes	Yes	13	8	21	80.9	3	43	44
9	F/50	8	No	No	Yes	14	3	17	44.6	1	31	31

normals versus 1.10 ± 0.09 for chronic back pain; $F = 4.71$, $P < 0.03$), lactate by 8.1%, glutamate by 7.2%, choline by 6.7%, and inositol by 6.2% (statistically significant where indicated). Thus, chronic back pain seems to be related to abnormal chemistry of the DLPFC. The thalamic glucose concentration was changed in the opposite direction. An increase of thalamic glucose by 11.7% was seen in chronic back pain subjects (mean ± SD 0.61 ± 0.04 for normals versus 0.68 ± 0.05 for chronic back pain; $F = 3.44$, $P = 0.06$). Cingulate and other brain regions including insula, sensorimotor, orbital frontal and visual cortices showed no differences in chemical concentrations between these two groups.

To ascertain whether chemical changes reflect part of a larger scale pattern for neurochemical organization, correlation analysis was used to determine the putative relationships between chemicals within and across brain regions. These chemical interrelationships, which we identify as 'chemical connectivity', were assessed for normals and chronic back pain patients. If the observed chemical changes in DLPFC in chronic back pain are a reflection of chemical connectivity with other brain regions, then the pattern of chemical interrelationships within and across brain regions should be different between the two groups. Correlation analysis for eight metabolites for three brain regions detected significantly high positive and negative correlations across 24 dimensions. This analysis revealed regional brain segregation across all chemical connectivity for the two groups of subjects. Fig. 3 shows the correlation matrices in normal controls and chronic back pain, within and across DLFPC, cingulate and thalamus. Each brain region occupies a specific quadrant within the matrix space. For both groups of subjects, the dominant positive correlations occur within brain regions and negative correlations are seen across brain regions. However, the pattern of chemical connectivity is different in chronic back pain subjects as compared to the normal subjects. In chronic back pain the positive chemical connectivity is weaker in the DLPFC, and the negative chemical connectivity is stronger between the thalamus and cingulate. We consider these changes in chemical connectivity an 'abnormal pattern'. A multivariate approach was used to test whether these two chemical connectivity patterns are statistically different. A four-way ANOVA of the strength of pairwise correlations

Table 2
Chemical findings in the nine patients with chronic low back pain across three brain regions[a]

Patient no.	N-Acetyl aspartate	Choline	γ-Aminobutyric acid	Glutamate	Glutamine	Glucose	Inositol	Lactate
Dorsolateral prefrontal cortex								
1	1.089	0.830	0.732	0.571	0.804	0.527	0.750	0.411
2	0.885	0.836	0.705	0.623	0.738	0.656	0.820	0.475
3	1.151	0.811	0.755	0.736	0.830	0.585	0.755	0.509
4	1.130	0.833	0.657	0.685	0.815	0.593	0.870	0.481
5	1.208	0.832	0.752	0.653	0.792	0.604	0.772	0.455
6	1.052	0.810	0.586	0.621	0.655	0.569	0.690	0.310
7	1.208	0.851	0.792	0.772	0.871	0.535	0.851	0.257
8	1.130	0.852	0.685	0.648	0.778	0.537	0.778	0.241
9	1.052	0.828	0.586	0.552	0.707	0.621	0.776	0.362
Thalamus								
1	0.918	1.000	0.545	0.573	0.673	0.682	0.836	0.291
2	1.184	0.932	0.524	0.573	0.505	0.660	1.010	0.427
3	1.173	0.952	0.692	0.673	0.750	0.731	0.962	0.404
4	1.070	0.789	0.491	0.474	0.561	0.684	0.737	0.386
5	1.109	0.909	0.600	0.600	0.709	0.727	0.873	0.382
6	1.130	0.907	0.667	0.574	0.741	0.630	0.833	0.333
7	0.984	0.951	0.574	0.590	0.623	0.574	0.795	0.328
8	1.074	1.000	0.481	0.500	0.593	0.685	0.870	0.352
9	1.196	0.902	0.549	0.549	0.627	0.745	0.784	0.373
Cingulate cortex								
1	1.082	0.857	0.735	0.684	0.724	0.816	1.000	0.469
2	1.132	1.154	0.692	0.747	0.857	0.747	1.341	0.527
3	1.298	0.809	0.553	0.532	0.755	0.766	0.766	0.404
4	1.245	1.051	0.714	0.694	0.898	0.776	0.918	0.449
5	1.184	0.932	0.718	0.699	0.796	0.699	0.660	0.427
6	1.130	0.907	0.630	0.685	0.796	0.574	0.870	0.537
7	1.109	0.964	0.764	0.727	0.945	0.782	0.955	0.436
8	1.196	0.980	0.725	0.667	0.843	0.549	0.804	0.490
9	1.196	0.863	0.569	0.608	0.784	0.569	0.843	0.392

[a] The relative concentrations of N-acetyl aspartate, choline, γ-aminobutyric acid, glutamate, glutamine, glucose, inositol and lactate were measured relative to the concentration for creatine/phosphocreatine complex.

Table 3
Chemical findings in the 11 normal subjects across the same three brain regions and eight chemicals as shown in Table 2 presented in relation to the concentration of creatine/phosphocreatine complex

Subject no.	N-Acetyl aspartate	Choline	γ-Aminobutyric acid	Glutamate	Glutamine	Glucose	Inositol	Lactate
Dorsolateral prefrontal cortex								
1	1.173	0.904	0.769	0.750	0.808	0.692	0.827	0.385
2	1.208	0.950	0.653	0.733	0.832	0.911	0.950	0.495
3	1.245	0.918	0.633	0.694	0.694	0.592	0.816	0.388
4	1.109	0.818	0.564	0.582	0.618	0.491	0.782	0.309
5	1.245	0.918	0.714	0.714	0.755	0.653	0.898	0.469
6	1.173	0.942	0.731	0.692	0.769	0.731	0.865	0.538
7	1.170	0.900	0.660	0.660	0.730	0.630	0.820	0.380
8	1.151	0.887	0.792	0.736	0.849	0.651	0.811	0.377
9	1.130	0.852	0.685	0.593	0.722	0.611	0.796	0.231
10	1.245	0.776	0.592	0.633	0.694	0.694	0.714	0.388
11	1.186	0.902	0.824	0.853	1.039	0.755	0.882	0.627
Thalamus								
1	0.934	0.951	0.508	0.459	0.574	0.533	0.836	0.344
2	1.089	0.857	0.554	0.643	0.696	0.607	0.804	0.429
3	1.184	0.990	0.660	0.621	0.777	0.602	0.835	0.408
4	1.052	0.879	0.534	0.500	0.603	0.569	0.879	0.328
5	1.017	0.900	0.567	0.550	0.617	0.683	0.850	0.317
6	1.220	0.920	0.640	0.560	0.640	0.620	0.940	0.300
7	1.196	0.980	0.667	0.627	0.745	0.608	0.843	0.431
8	1.173	0.942	0.538	0.558	0.692	0.635	0.788	0.365
9	1.130	1.037	0.685	0.648	0.722	0.685	0.852	0.426
10	1.070	0.982	0.614	0.561	0.614	0.596	0.772	0.351
11	1.151	0.915	0.547	0.585	0.717	0.623	0.830	0.321
Cingulate cortex								
1	1.132	1.000	0.755	0.717	1.000	0.736	0.887	0.302
2	1.220	0.900	0.760	0.660	0.760	0.800	0.980	0.420
3	1.130	1.019	0.704	0.685	0.870	0.593	0.889	0.222
4	1.053	0.965	0.754	0.702	0.737	0.702	0.912	0.404
5	1.112	0.857	0.755	0.735	0.837	0.673	0.939	0.571
6	1.151	0.887	0.698	0.698	0.849	0.698	0.962	0.396
7	1.160	0.970	0.720	0.690	0.830	0.670	0.880	0.410
8	1.245	0.959	0.694	0.612	0.735	0.612	0.776	0.429
9	1.142	1.047	0.792	0.698	1.019	0.717	0.925	0.566
10	1.089	0.821	0.768	0.714	0.804	0.679	0.750	0.536
11	1.140	1.019	0.617	0.579	0.766	0.617	0.935	0.393

revealed a main effect for location (within a region versus between regions) ($F(1, 1056) = 294.77$, $P < 10^{-7}$), brain region ($F(2, 1056) = 6.97$, $P < 0.001$), diagnosis ($F(1, 1056) = 5.00$, $P < 0.03$), the location × chemical interaction ($F(7, 1056) = 4.72$, $P < 0.00003$), the location × brain region interaction ($F(2, 1056) = 5.66$, $P < 0.004$), the diagnosis × brain region interaction ($F(2, 1056) = 4.14$, $P < 0.02$), the location × chemical × brain region interaction ($F(14, 1056) = 2.52$, $P < 0.002$), and the location × brain region × diagnosis interaction ($F(2, 1056) = 11.37$, $P < 0.00001$).

The observed chemical connectivity differences suggest a specific hypothesis based on univariate statistics to identify the diagnosis of each subject (random effects model). One such statistic is the mean/SD across all chemicals for each brain region calculated for each subject after transforming concentrations into Fisher's Z values. This statistic applied to DLFPC distinguishes between the two groups of subjects ($F(10, 9) = 4.21$, $P < 0.02$). The same statistic applied to the cingulate or thalamus fails to make the diagnostic distinction. A second univariate statistic is the cross product of glucose and glutamate in the DLPFC subtracting the cross product of N-acetyl aspartate in the thalamus and glutamine in cingulate after Z-transforming concentrations. This hypothesis is based on the largest differences seen in chemical connectivity patterns (Fig. 3), and this too differentiates between the two groups of subjects ($F(10, 9) = 3.96$, $P < 0.03$).

If the observed abnormal chemical connectivity pattern in chronic back pain is specific for this diagnostic group, then the regional chemical variations should be related to the perceptual measures of pain and anxiety. The correlations of these regional chemicals with 23 perceptual descriptors are shown in Fig. 4. We used a multivariate approach to test whether this chemical–perceptual network in chronic back pain is statistically different across brain regions and

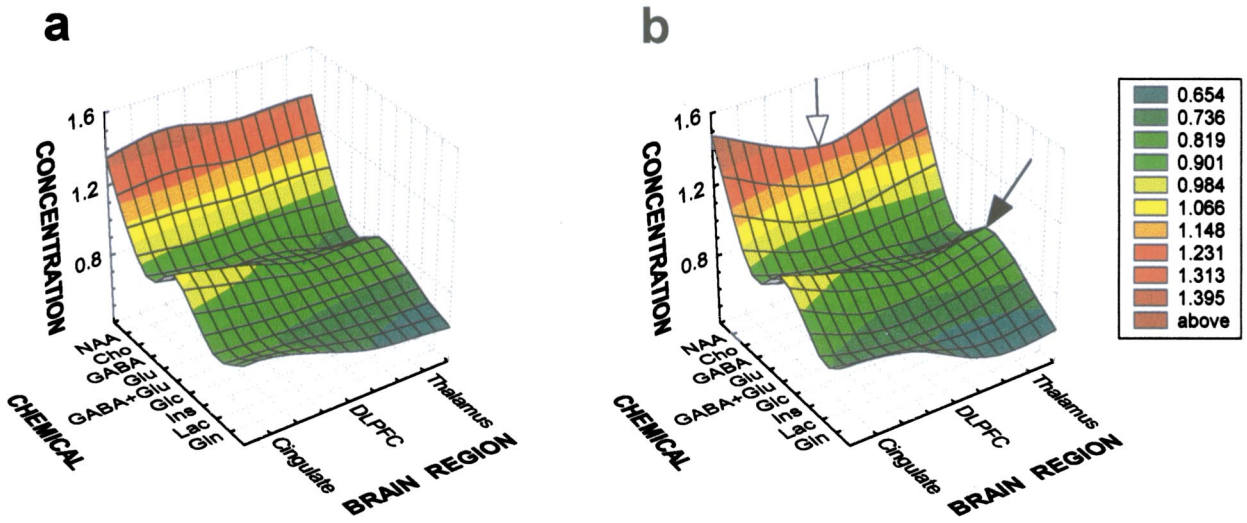

Fig. 2. Chemical concentration differences between normal controls (a) and chronic back pain (b) in three regions of the human brain. Relative concentrations of N-acetyl aspartate, choline, GABA, glutamate, GABA plus glutamate, glucose, myo- and scyllo-inositol complex (inositol), lactate, and glutamine are shown. The concentrations of N-acetyl aspartate (white arrow) and glucose are lower in the DLPFC and the glucose level is higher in the thalamus (black arrow) in chronic back pain, as compared with the same regional metabolites in normal controls. These surface plots are a visual aid; they do not imply continuity between chemicals or brain regions.

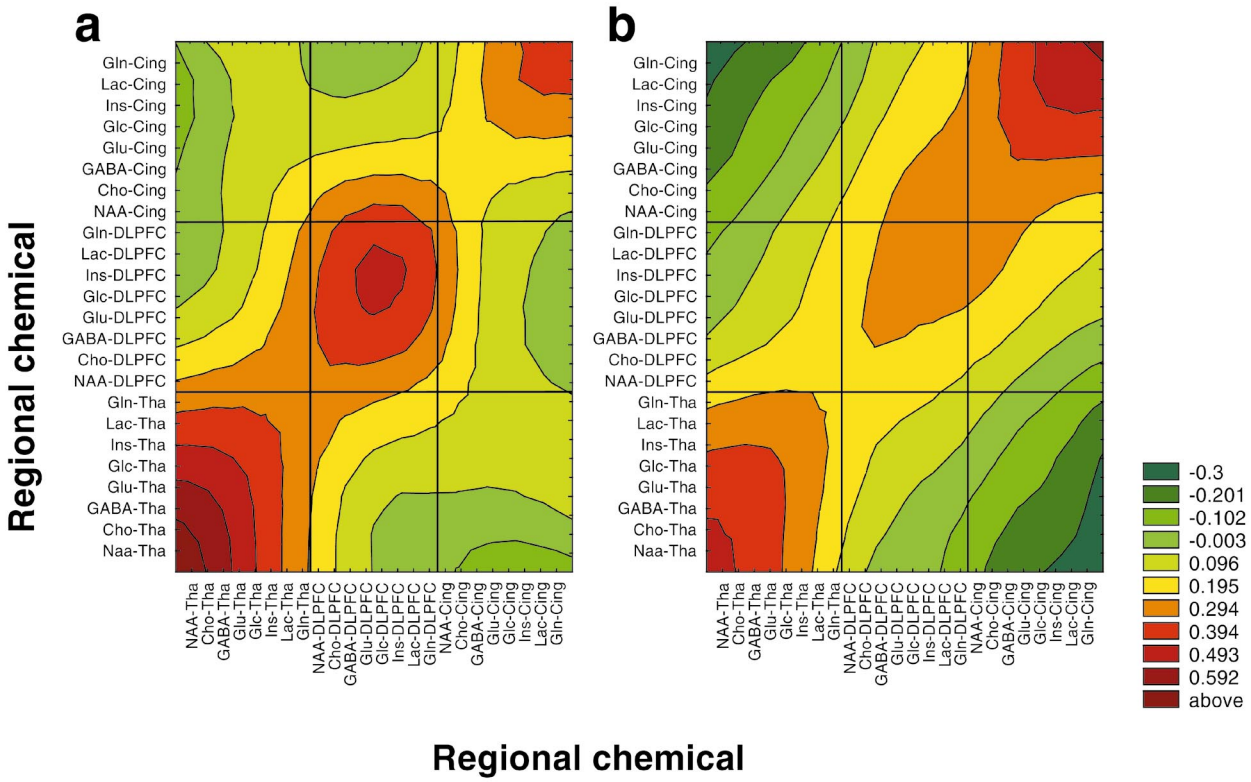

Fig. 3. Contour plots showing within and across regional correlations of chemical concentrations in normal controls (a) and chronic back pain subjects (b). The 24 regional metabolites are labeled by chemical region (eight metabolites for three brain regions: Tha, thalamus; DLPFC, dorsolateral prefrontal cortex; Cing, cingulate; metabolites are labeled as in Fig. 1). The correlation values (Pearson's correlation coefficient) are presented as color gradients (red, positive; green, negative). In normal subjects strong positive correlations are seen mainly within brain regions, most prominently in DLPFC. In chronic back pain positive correlations are weaker within the DLPFC, and negative correlations are stronger between the thalamus and cingulate.

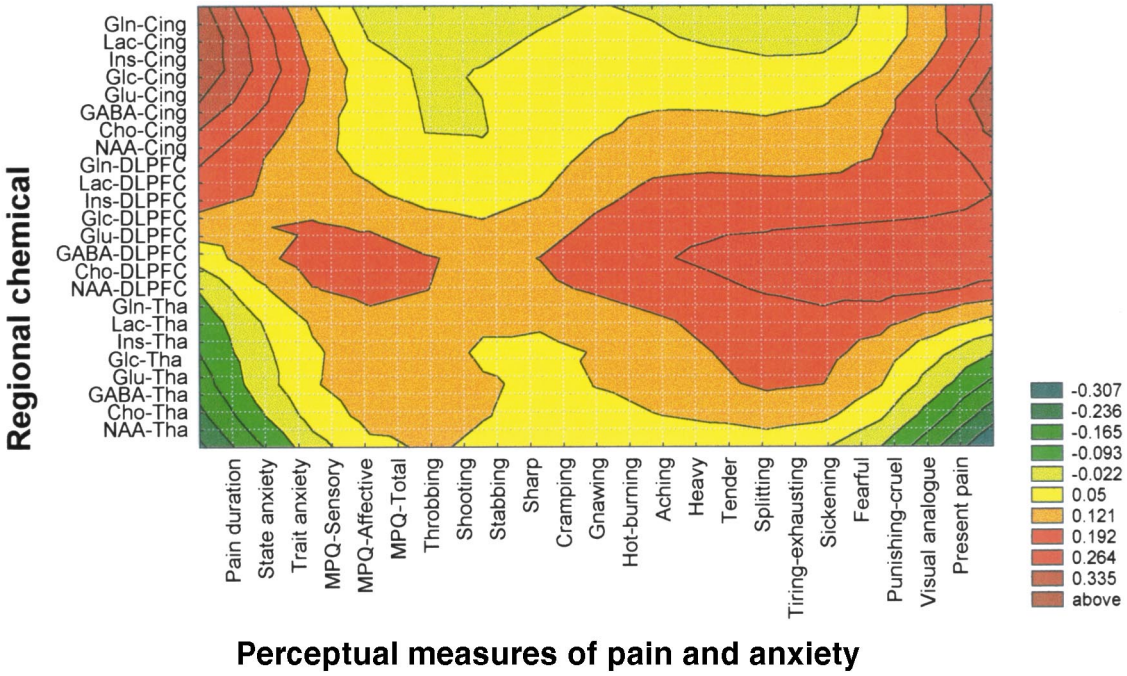

Fig. 4. Contour plot of correlations between regional chemical concentrations and perceptual measurements of chronic back pain. The correlations of the same 24 regional chemicals as shown in Fig. 3 with 23 perceptual descriptors for pain and anxiety are presented. The strongest positive correlations are between chemicals in DLPFC and behavioral measurements.

pain/anxiety perception. Analysis of variance of the strength of pairwise correlations revealed a significant main effect for pain/anxiety perception ($F(22,483) = 1.71$, $P < 0.02$), brain region ($F(2,483) = 13.52$, $P < 0.000002$), and the pain/anxiety perception × brain region interaction ($F(44,483) = 1.90$, $P < 0.0007$, two-way ANOVA). The post-hoc comparison demonstrated the strongest regional effect for DLPFC ($F = 55.70$, $P < 10^{-7}$). The mean strength of correlations between DLPFC and perceptual measures of pain and anxiety is nine times stronger than the strength of correlations for cingulate (mean $r = 0.18$ for DLPFC versus $r = 0.02$ for cingulate, $P < 0.00002$; post-hoc Sheffe test), and six times stronger than for thalamus (mean $r = 0.03$, $P < 0.0001$ as compared to mean r in DLPFC). Each perceptual descriptor occupies a specific area within the matrix space. The sensory, affective, and total components of the SF-MPQ are linked with the state and trait anxiety levels (all $r = 0.80$–0.83, $P < 0.05$ for the r values). However, the duration of pain (i.e. pain chronicity) is negatively related to the affective component of the SF-MPQ ($r = -0.68$, $P < 0.05$), and unrelated to anxiety, present pain index, and visual analogue scale ($r = -0.13$ to -0.36). Post-hoc comparisons revealed specific functional relationships between each of the perceptual descriptor and regional metabolites. The state and trait anxiety was found to be related to prefrontal and cingulate chemicals ($F = 9.19$, $P < 0.003$; comparing r values for eight chemicals for the two anxiety measures in these two regions to those in the thalamus). The duration of pain is related to cingulate chemicals ($F = 7.27$, $P < 0.007$; comparing cingulate r values to the other two regions), and the sensory, affective, and total components of the SF-MPQ are related to prefrontal metabolites ($F = 16.66$, $P < 0.00005$; comparing DLPFC r values to the other two regions), while the present pain intensity index is related to both prefrontal and cingulate chemicals ($F = 13.51$, $P < 0.0003$; r values compared between them and the thalamus) with the strongest link to prefrontal metabolites ($F = 6.65$, $P < 0.01$; r values compared between DLPFC and the other two regions). Each pain descriptor of SF-MPQ is related to prefrontal chemicals (all $P < 0.00003$; comparing r for all pain descriptors in this region to the other two regions), with a strength increase toward the affective versus sensory components ($F = 32.49$, $P < 10^{-7}$; comparing r of the first 11 descriptors to the last four in DLPFC). However, within the sensory descriptors of chronic back pain, the cramping, gnawing, hot-burning, aching, heavy, tender, and splitting are more strongly related to prefrontal metabolites than shooting, stabbing and sharp pain ($F = 17.97$, $P < 0.00003$). These findings indicate that the abnormal pattern of chemical connectivity in chronic back pain, which we described above, is related to pain perception.

We used stepwise multiple linear regression (backward elimination) analyses to predict relationships between concentration levels of three regional chemicals (prefrontal N-acetyl aspartate and glucose, and thalamic glucose), which were found to be abnormal in chronic back pain patients, and the 23 perceptual measures for pain and anxi-

ety. Highly significant empirical relationships were seen between six perceptual predictors and these three regional chemicals: (1) the combination of sharp pain, stabbing pain, pain duration, and trait anxiety predict the concentration of DLPFC N-acetyl aspartate ($R^2 = 0.98$, $F(4,4) = 43.77$, $P < 0.001$); (2) the combination of sharp pain, stabbing pain, sensory component of the SF-MPQ, and trait anxiety predict the concentration of DLPFC glucose ($R^2 = 0.99$, $F(4,4) = 98.56$, $P < 0.0003$); (3) the combination of pain duration, and state and trait anxiety predict the concentration of thalamic glucose ($R^2 = 0.97$, $F(3,5) = 46.72$, $P < 0.0004$). Overall, this analysis suggests the presence of specific relationships between the regional chemicals tested and pain perception.

4. Discussion

In this report we provide direct evidence for brain chemical abnormalities in chronic back pain, and demonstrate that chronic pain perception is linked to a specific pattern of chemical network most likely as a result of long-term cortical reorganization. However, the mechanisms of this chemical network reorganization in chronic pain, and understanding the role of specific neurotransmitter changes in pain pathophysiology, remain to be examined.

Our findings also might explain the low diagnostic value of structural neuroimaging studies (i.e. MRI) of the lumbar spine in this category of subjects. Local disk abnormalities might play a certain role in acute cases of back pain as a trigger of pain mechanisms, and when the process becomes chronic other more central mechanisms driven by or causing changes in brain chemistry may be more important. If this is the case, ^{1}H-MRS of the brain, rather than MRI of the lumbar spine, will play a major role in diagnosis of chronic back pain and monitoring of surgical and non-surgical treatments and outcomes. The proper documentation of brain chemical changes in this category of patients may accelerate future development of more effective pharmacological treatments, which has not been successfully accomplished in the past.

Our data suggest that depletion of N-acetyl aspartate and glucose in prefrontal cortex can be considered as a landmark of chronic pain. It is known that N-acetyl aspartate is localized within neurons and involved in synaptic processes, and can be considered as a neuronal and axonal marker (Miller, 1991; Castillo et al., 1998). Subsequent breakdown of N-acetyl aspartate leads to aspartate, which is an excitatory amino acid neurotransmitter. Decreases in N-acetyl aspartate have been documented in various conditions involving neuronal cell damage and loss, including stroke, multiple sclerosis, Alzheimer's disease, epilepsy, and several neurodegenerative disorders (Salibi and Brown, 1998), suggesting that our results provide evidence for a link between chronic pain and neuronal loss and degeneration. N-Acetyl aspartate may be a more sensitive marker of neuronal loss in the brain than structural MRI (Guimaraes et al., 1995; Winsberg et al., 2000). Glucose is commonly considered as a marker of the general metabolic rate, and is involved in the metabolic pathway of the tricarboxylic acid cycle of neurons and astrocytes (Gruetter et al., 1996; Shulman and Rothman, 1998; Magistretti et al., 1999). Within the brain glucose is processed glycolytically, resulting in the release of lactate as an energy substrate for neurons (Magistretti et al., 1999). The energy demands of glutamatergic neurons account for 80–90% of total cortical glucose usage (Sibson et al., 1998). This might explain the reduction of lactate and glutamate in the DLPFC of chronic back pain subjects (not significant in this study). It is not clear why the observed differences in glucose concentration in the DLPFC and thalamus are changed in opposite directions. This may reflect functional inhibitory reciprocal connections for these two regions (Fuster, 1997).

In a previous study of normal volunteers aged 19–31 years we demonstrated the existence of a chemical network in the brain (Grachev and Apkarian, 2000b). Here we show a similar chemical network for 40–52-year-old volunteers, which is disrupted in the same age chronic pain patients. From this viewpoint, the specific chemical concentration changes that we observe may be a reflection of the disruption of the network. The disruption of the network in chronic pain seems specific. Within region chemical connectivity decreased (mainly in DLFPC), while across region connectivity increased (i.e. negative correlations are strengthened). The abnormal pattern of chemical connectivity shows a well-defined relationship to the perceptual attributes of the chronic pain. This relationship shows that the chemical network reflects the functional/perceptual state of the brain, linking neurochemistry to pain/anxiety perception. The specific chemical relationships to perception of pain and anxiety again may reflect the properties of the network.

Previous fMRI studies of chronic pain revealed engagement of the prefrontal cortex (Rosen et al., 1994; Hsieh et al., 1996; Apkarian et al., 2000). The most recent fMRI study of brain activity and behavioral performance in normal subjects shows that the prefrontal cortex is related to awareness in sensory learning (McIntosh et al., 1999). Our results here provide chemical evidence for the awareness of chronic pain occurring in the prefrontal cortex, which in turn may disrupt other cognitive processing, as we have shown previously (Apkarian et al., 2000). Moreover, the prefrontal relationship to awareness engages a network of diverse brain regions (McIntosh et al., 1999). Here we show that the chemical changes associated with chronic pain also occur across a large-scale network within the brain. In conclusion, our results provide direct evidence of abnormal brain chemistry and chemical network in chronic back pain, which may be a consequence of long-term neurotransmitter changes in chronic pain sufferers. Studies in larger cohorts where non-specific confounders such as medications and motor disabilities could be taken into account, as well as examining the right hemisphere,

are needed to replicate these initial data. Future studies will be important for understanding whether or not the observed differences in the brain neurochemistry in chronic back pain can be generalized for other pain states and whether these brain chemical abnormalities may be reversed following pain alleviation (see e.g. Hugg et al., 1996; showed normalization of Cr/NAA in the unoperated contralateral tissue in patients with temporal lobe epilepsy following surgical elimination of seizures). The tantalizing conclusion that chronic pain may be associated with neural degeneration needs to be re-examined with morphometric analysis.

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