Using Arterial Spin Labelling to Investigate Spontaneous and Evoked Ongoing Musculoskeletal Pain.

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Abstract

Clinical pain is difficult to study using standard blood oxygenation level dependent (BOLD) imaging. Arterial spin labelling (ASL) offers an attractive alternative as it can measure perfusion during a state or a prolonged stimulation. We propose a paradigm which uses arm repositioning to evoke clinically-relevant musculoskeletal pain in patients with shoulder impingement syndrome. We also present an analysis pipeline using tools from the FMRIB Software Library (FSL) optimised for analysis of perfusion images of middle-aged and elderly patients. Patients with shoulder pain and healthy controls were scanned using multi post-labelling delay pseudo-continuous ASL (pCASL), initially in the supine position and then with one arm raised to evoke clinical pain. The proposed paradigm resulted in increased perfusion in the patient group in cortical and subcortical regions known to be involved in sensory processing and movement integration, such as the ipsilateral primary somatosensory cortex, ipsilateral operculum/insular cortex, ipsilateral putamen and bilaterally in the thalamus, midbrain, and the cerebellum. Perfusion changes were not present in the control group, which suggests that they were related to pain rather than just arm repositioning. The optimised analysis improved registration of perfusion images in comparison to standard approaches.

Key words: fMRI, ASL, FSL, registration, musculoskeletal pain, chronic pain

Word count: 5239

INTRODUCTION

Some conditions cannot be easily studied using standard Blood Oxygenation Level Dependent (BOLD) functional magnetic resonance imaging (FMRI). For example, clinical pain is usually either spontaneous and ongoing or, when evoked, it diminishes too slowly to be used in an event-related or block design. Moreover, clinical musculoskeletal pain, especially shoulder pain, is challenging to image using a traditional BOLD FMRI paradigm because the movement of the affected arm so close to the head dramatically impacts the shim of the scanner and potentially causes changes in head position resulting in significant artefacts; independently of the high likelihood of causing stimulus-correlated motion artefacts.

There have been attempts at using BOLD FMRI to study ongoing pain by correlating brain activation with a continuous rating of fluctuating levels of pain [1,2]. However, Howard et al. argued that these studies examined correlation between the brain activation and pain ratings rather than pain itself, and that the results were confounded by the constant appraisal of pain and motor response related to pain rating [3].

Arterial spin labelling (ASL) offers an attractive alternative to BOLD FMRI. ASL has been used to measure perfusion changes related to experimental pain in healthy people [4,5] as well as clinical pain in chronic pain patients [6,7]. Pain-related perfusion was analysed either by comparing brain perfusion during a painful and pain-free state, between patients and controls [3,6] or by performing time-course analysis by correlating the pain ratings during imaging with the perfusion values [5,7].

Experimentally induced pain is easier to study than clinical pain because it can be transient, standardised, reproducible and still provide useful information about pain

mechanisms; however, it is the clinically-relevant pain that is of primary interest in trials of treatment efficacy[8,9]. Some types of clinical pain, such as pain after tooth extraction, can be reliably evoked in all patients [3], whereas other types of pain are more difficult to investigate. For example, one study had to exclude 7 out of 27 recruited patients because their clinical pain did not diminish quickly enough to obtain a pain-free baseline for the experimental pain session [6]. Another study excluded 9 out of 43 patients because they reported no pain on the day of the scan [7]. Losing recruited patients is a significant problem considering how difficult it is to run and recruit pain patients into longitudinal studies.

Aims and hypotheses

In this paper, we proposed a paradigm to study brain perfusion during spontaneous and evoked ongoing clinical musculoskeletal pain. The hypothesis was that raising of the affected arm would evoke pain in shoulder pain patients but not in controls and would be associated with increased perfusion in pain-processing regions in patients but not in controls (within-group) as well as differences in perfusion between patients and controls when scanned with the affected arm up and possibly also when the arm is resting along the body (between groups). We also expected that these changes in perfusion would correlate with reported pain ratings. In this paper, we also presented an improved registration of ASL data to a standard template, which is particularly problematic in the case of ASL data due to a poor tissue contrast and lack of anatomical details.

METHODS

Participants

Shoulder pain patients participating in the CSAW trial were invited to take part in a neuroimaging study. The CSAW trial (Can Shoulder Arthroscopy Work, NCT01623011) investigated the efficacy of arthroscopic decompression surgery for chronic shoulder impingement pain. The inclusion criteria for patients were: subacromial pain due to tendinopathy or partial tear lasting at least three months despite conservative treatment and lack of contraindication for MRI. A detailed list of inclusion and exclusion criterial were described in the trial protocol [10].

Healthy control participants were recruited using advertisements within the Oxford hospitals. Volunteers were eligible if they were healthy, pain-free, and had no contraindications for MRI.

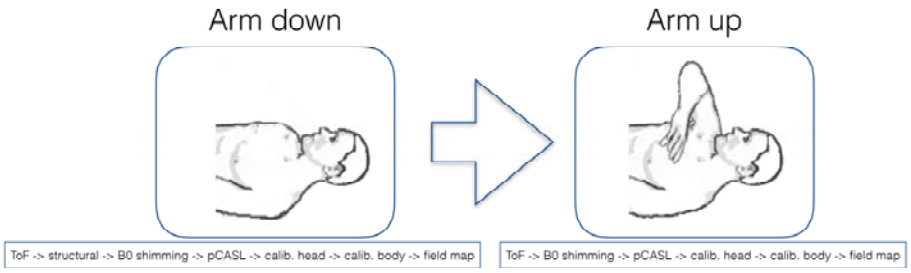
This study was performed according to the Declaration of Helsinki and approved by the local Research Ethics Committee (REC Reference 12/sc/0028).

Experimental design

Two ASL datasets were collected for each subject. Firstly, patients were scanned laying in a supine position with their arms along the body (arm-down condition) to measure cerebral perfusion during a pain-free state or during spontaneous ongoing pain (depending on whether patients experienced spontaneous ongoing pain at rest). During the subsequent scan, patients were asked to move the affected arm into a position, which evoked ongoing clinical pain, (arm-up condition) i.e., maximal abduction and lateral rotation within the scanner constraints (Fig. 1). This scan measured cerebral perfusion during ongoing evoked clinical pain. As this was the last scan of the session, patients were advised to press the buzzer if the pain became

intolerable, which prompted an immediate termination of the scan and the imaging session. Controls were also scanned with their arm down and up, in a similar position as patients. The later scan was used to control for the effects of arm repositioning and the side was randomised.

Fig. 1 Scanning conditions



The arm-down scans were acquired independently from the arm-up scans. During the arm-down scan, a localiser, a time-of-flight scan and a high-resolution structural scan were performed first. The time when the structural image was being acquired, was used to identify the labelling plane and plan the ASL scan. The labelling plane was chosen based on the time-of-flight scan of the neck arteries; it was positioned perpendicular to the axis of the vessels, at approximately the level of the second cervical vertebra [11]. This was followed by the main ASL scan, two calibration scans (first using the head coil, then the body coil for signal reception), and a field map scans. After this series of scans, patients were asked to verbally rate their average pain during the arm-down ASL scan on a 11-point numerical rating scale (NRS) with the anchors being 0="no pain" and 10="worst pain possible". Between the arm-down and the arm-up scan, the participant was repositioned. The raised arm was padded with foam wedges for support and to prevent movement and to provide insulation from the side of the scanner. The arm-up scanning procedure consisted of re-shimming, new localizer and time-of-flight scans, re-planning the location of the tagging plane, and a new set of ASL, calibration and field map scans. After these

scans, the patient was removed from the scanner and asked to verbally rate their average pain during the arm-up scan.

Imaging parameters

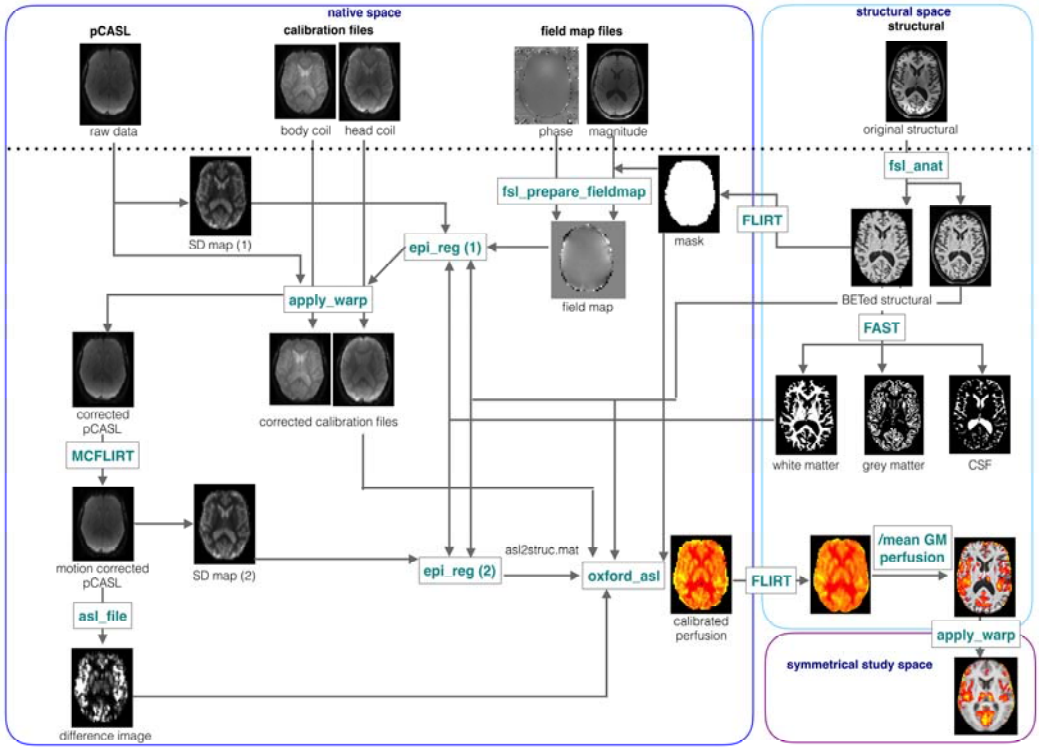
Neuroimaging was performed on a 3T Verio MRI scanner (SIEMENS, Erlangen, Germany) using a 32-channel head coil. We used pseudo-continuous ASL (pCASL) with background suppression (using pre-saturation) and an EPI readout [11]. The sequence parameters were: repetition time (TR) 4000ms, echo time (TE) 13ms, Partial Fourier=6/8th, flip angle 90^{o}, FOV 240x240m, matrix 64x64. There were 24 slices collected in ascending order. The in-plane resolution was 3.75x3.75mm^2, and the slice thickness was 4.5mm with a 0.5mm gap. There were 6 post-labelling delays, 250ms apart (250, 500, 750, 1000, 1250, and 1500ms) with label duration 1400ms and extra post-labelling delay for superior slices of 45.2ms per slice. These parameters were validated for modelling of kinetic curves across the whole brain in human subjects [11], including pain studies [5]. There were 120 volumes (10 epochs of 6 label-control pairs) collected in just over 8 minutes.

Two calibration images were acquired without background suppression or labelling, one using the head coil and the other using the body coil for signal reception. The TR was 6000ms and all the other parameters were the same as for the main pCASL sequence. Field maps were collected with the same orientation and voxel size as the pCASL and TR=400ms, TE_1=5.19ms, TE_2=7.65ms, flip angle 60^{o}. We also acquired a structural image using the 3D MPRAGE sequence with the following parameters: TR=2040ms, TE=4.7ms, TI=900ms, flip angle 8^{o}, FOV read 192mm, 1mm isometric voxels.

Analysis pipeline

The data were analysed using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) tools version 5.0.9 (http://fsl.fmrib.ox.ac.uk) and Matlab (Mathworks, Natick, MA, USA).

Fig. 2 Pre-processing pipeline



An overview of the multi-stage analysis pipeline is presented in Fig. 2. It is also described in detail in Appendix 1 and the script is provided as supplementary material. In brief, the non-brain tissue was masked out and *epi_reg,* with an ASL variance map as a reference image was used to correct the ASL data and calibration files for B_0 distortions and to create a good transform between the native ASL space and the structural space. ASL data were motion-corrected using *MCFLIRT* [12]. Then, the label-control pairs were subtracted and the result averaged at each post-label delay using asl_file and quantified using oxford_asl., which are a part of the BASIL

toolbox (Bayesian Inference for Arterial Spin Labelling MRI) [13]. The voxel-wise calibration option was used, as recommended in the white paper [15], to correct for possible change in coil sensitivity due to arm repositioning [14].

The individual perfusion images were transformed to a study-specific template using an affine (FLIRT) and then nonlinear (FNRIT) registration, in standard FEAT analysis, and smoothed with a sigma 3mm (FWHM=7.05 mm) Gaussian kernel before the group analysis.

Statistical analysis of non-imaging data

Baseline characteristics in the patient and control group were compared using two-sample t-tests for continuous variables and Fisher's exact tests for categorical variables.

A regression analysis was used to analyse differences in mean grey matter perfusion and arterial transit time (ATT) between patients and controls and between arm-down and arm-up condition, as well as interactions between those two factors, controlling for age, sex and clustering within each subject.

Statistical analysis of non-imaging data was performed using STATA version 12.1 [16].

Statistical analysis of imaging data

Within-group differences between the arm-up and arm-down condition were analysed, separately within the patient and control group, using a voxel-wise paired t-test with the demeaned mean grey matter perfusion values for each participant entered as a covariate of no interest. In the patient group, the analysis was repeated with demeaned individual pain ratings as an additional covariate. A one-sample t-test with demeaned pain ratings as well as demeaned age, sex and the mean grey matter

perfusion was also performed in the patient group, for the arm-down and arm-up condition separately.

The between-group differences (patients versus controls) were investigated using a two-sample test (with age and sex as covariates of no interest), for the arm-down and the arm-up condition separately.

The analysis was also carried out after flipping the perfusion images along the x-axis for participants who were scanned with the left arm up.

Statistical analysis of imaging data was performed using *randomise* [17] with 5000 permutations, corrected for family-wise error using Threshold-Free Cluster Enhancement (TFCE) and thresholded to show voxels significant at $p<0.05$. The analysis was limited to voxels within the grey matter mask common to all the datasets.

Post hoc analysis

We also performed a post hoc region of interest (ROI) analysis to quantify the observed perfusion changes. We extracted mean perfusion values from a sphere around the maximum voxel in activation clusters identified in the within-group analysis of x axis flipped data. We chose the flipped analysis so that the activation could be described as either ipsi- or contralateral.

The mean perfusion values were entered into a regression model along with the group (patients/controls), condition (arm-up/arm-down), age, sex, and the mean grey matter perfusion and clustered by subject. The analysis was performed for each ROI separately.

The effect of condition and reported pain intensity was analysed only in the patient group using a regression model with ROI perfusion, age, sex, the mean grey matter perfusion, and condition, clustered by subject.

RESULTS

Participants' characteristics

67 patients participated in the neuroimaging part of the CSAW trial. ASL data was not collected from five subjects (due to MRI contraindications, incidental findings, anxiety, or excessive pain during the scanning session unrelated to the evoked pain), and seven datasets were discarded due to technical reasons (incomplete brain coverage or missing calibration files). Two patients pressed the buzzer during the arm-up scan, which resulted in acquisition of 8 out of 10 epochs. These two datasets were included in the analysis as they did not differ from other datasets in perfusion pattern or mean and variance of grey matter perfusion. Twenty-four healthy controls were recruited; however, one could not be scanned due to MRI contraindications, one had incidental findings, one dataset was discarded due to excessive motion, and one due to technical problems. This left 55 patient and 20 control datasets for analysis.

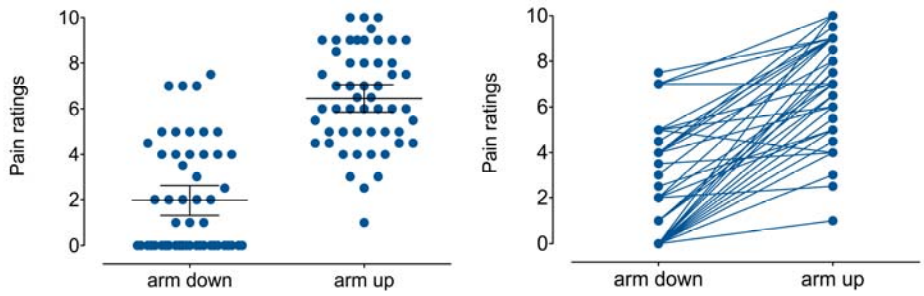
Patients and controls groups were matched in terms of age and sex. Mean age in the patient group was 51.5 years (SD 11.4, 95%CI 48.4 to 54.6) and 48.7 years in the healthy control group (SD 8.7, 95%CI 44.6 to 52.8), and the difference between the mean age was not statistically significant (t=-0.998 p=0.32). The male to female ratio was similar in both groups: 36% female in the patient group and 40% female in the control group (p=0.79). There were also no differences between which arm was raised during the arm-up scan: 39 out of 55 patients had right arm affected (right arm

up during the arm-up condition) and 11 out of 20 controls raised their right arm during the arm-up condition (p=0.27).

Pain ratings in the patient group

The mean pain rating on the NRS for arm-down was 1.96 (SD 2.4, 95%CI 1.3 to 2.6) and for arm-up 6.5 (SD 2.2, 95%CI 5.9 to 7.0) (Fig. 3) and the difference was statistically significant (t= -13.6 p<0.00005). The arm-down condition was painful for 51% of the patients and the arm-up condition was painful for all patients; neither condition was painful for healthy controls.

Fig 3 Ratings for pain intensity on a numerical rating scale (NRS) during arm-down and arm-up condition in the patient group (mean with 95% confidence interval) and change in ratings between arm-down and arm-up condition for each patient.



Optimised registration

This approach resulted in an improved registration in comparison to FLIRT or *epi_reg* with calibration images used as a reference. The mean cost function across all subjects for standard registration of a calibration image to structural image using FLIRT was 0.20 (95%CI 0.18 to 0.22), the mean cost function for registration of calibration image to the structural image using *epi_reg* with field map correction was

0.17 (95%CI 0.16 to 0.19) and the mean cost function for the optimised registration using *epi_reg* with variance map as an input was the lowest: 0.16 (95%CI 0.15 to 0.18).

Mean grey matter perfusion per condition

The mean grey matter perfusion in the patient group during arm-down condition was 64.93 ml/100g/min (SD 10.9, 95%CI 61.98 to 67.89) and during arm-up 63.23 ml/100g/min (SD 12.24, 95%CI 59.93 to 66.53); in the control group, mean grey matter perfusion during arm-down was 62.28 ml/100g/min (SD 10.01, 95%CIs 57.59 to 66.96) and during arm-up 61.18 ml/100g/min (SD 10.20, 95%CI 56.41 to 65.96).

The mean perfusion within the grey matter did not differ between patients and controls (β=4.07 ml/100g/min, 95%CI -0.64 to 8.74) or between the arm-up and arm-down condition (β =-1.1 ml/100g/min, 95%CI -3.07 to 0.88) when tested using a linear regression clustered by subject, adjusted for age and sex. The interaction between group and condition was also not significant (β=-0.60, 95%CI -3.1 to 1.9). Both older age (β =-0.34 ml/100g/min, 95%CI -0.51 to -0.17) and male sex (β =-12.6 ml/100g/min, 95%CI -16.5 to -8.6) were associated with lower perfusion.

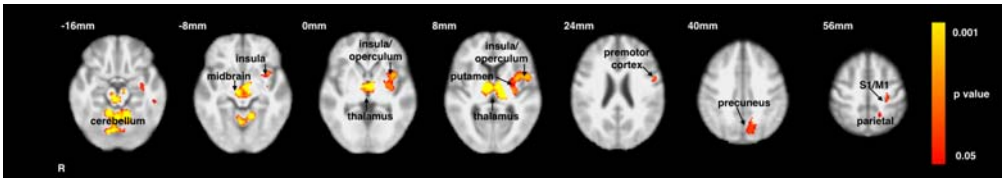
The arterial transit time (ATT) values also did not differ for patients in comparison to controls (β=-0.035s, 95%CI -0.07 to 0.002) and for arm-up in comparison to arm-down position (β=-0.001s, 95%CI -0.01 to 0.01), as analysed using linear regression with age and sex as covariate and clustering by subject.

Perfusion differences between arm-up and arm-down condition

In the patient group, there was an increase in perfusion between the arm-up and arm-down condition in the left primary sensorimotor cortex, a cluster encompassing

the left insular cortex, left operculum and the left putamen, the left amygdala, a large cluster including bilateral changes in the thalamus, midbrain, and cerebellum (Fig. 4). Details of peak value within significant clusters are presented in Table 2.

Fig 4 Within-group differences between arm-up and arm-down condition



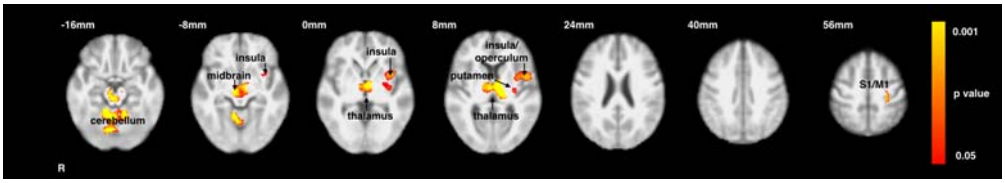
Paired t-test in 55 patients comparing arm-up > arm-down condition. The statistical image was FWE-corrected using threshold-free cluster enhancement (TFCE) and thresholded to show voxels significant at $p<0.05$. Abbreviations: S1 – primary somatosensory cortex, M1 – primary motor cortex.

Table 2 Maximum p-values within each cluster for the comparison presented in Fig. 4.

Voxels	p-max	X (mm)	Y (mm)	Z (mm)	Location
8092	0.999	-34	-56	-28	cerebellum
900	0.986	-50	4	8	left operculum
313	0.973	-10	-58	42	precuneus
231	0.965	-34	-8	-22	left amygdala
180	0.967	-24	-24	60	left primary sensorimotor cortex (S1/M1)
33	0.957	-56	-34	-24	left temporal gyrus
27	0.961	-24	-46	70	left superior parietal lobule

When the within-group analysis was performed after flipping the smoothed perfusion images along the x-axis for the participants who were scanned with the left arm up, the activation pattern (Fig. 5) was similar to the original analysis (Fig. 4) but some of the smaller clusters in the precuneus and amygdala were no longer significant (Table 3).

Fig 5 Within-group differences between arm-up and arm-down condition; flipped along the x-axis



Paired t-test in 55 patients comparing arm-up > arm-down condition; flipped along the x-axis so that the right side of brain was the ipsilateral side. The statistical image was FWE-corrected using threshold-free cluster enhancement (TFCE) and thresholded to show voxels significant at p<0.05. Abbreviations: S1 – primary somatosensory cortex, M1 – primary motor cortex.

Table 3 Maximum p-values within each cluster for the comparison presented in Fig. 5.

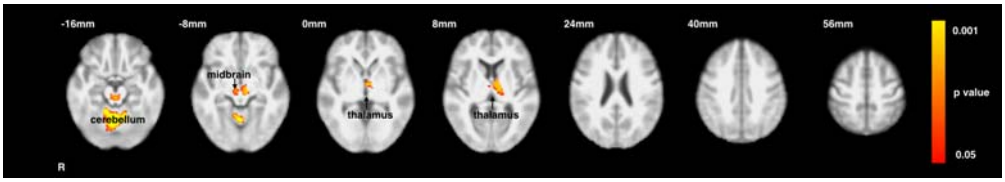
Voxels	p-max	X (mm)	Y (mm)	Z (mm)	Location
826	0.999	-38	-50	-28	left cerebellum
566	0.986	-50	4	8	left operculum
362	0.987	-26	-26	56	left primary sensorimotor cortex

In the control group, the paired t-test between the arm-up and arm-down condition did not result in significant clusters, even after flipping the images along the x-axis. The opposite contrast showed (at p<0.05) a small cluster in the right occipital cortex which was likely to be an artefact.

Correlations with pain ratings in the patient group

In the patient group, a paired t-test between perfusion during the arm-up and arm-down condition, with individual pain ratings and the mean grey matter perfusion as covariates, neither the difference between the conditions nor the pain ratings resulted in a significant but an F-test of these variables did (Fig. 6). The significant cluster extended from thalamus, through midbrain to the cerebellum with the peak voxel in the left cerebellum (maximum p-value coordinates in mm: -34,-62,-36).

Fig 6 F-test for within-group differences and pain ratings in a paired t-test between arm-up and arm-down condition in patients, on data flipped along the x-axis



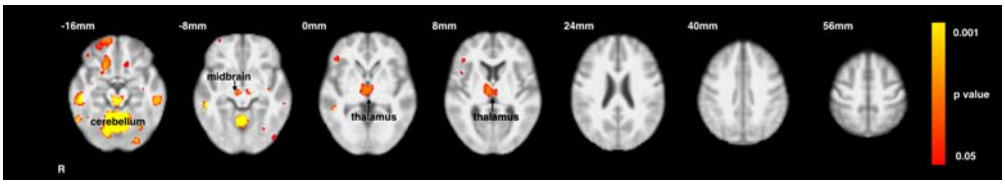
F-test results for an effect of arm-up versus arm-down condition and pain ratings in a paired t-test in 55 patients comparing arm-up > arm-down condition, flipped along the x-axis for patients with affected left arm, with pain ratings and mean grey matter perfusion as covariates. The statistical image was FWE-corrected using threshold-free cluster enhancement (TFCE) and thresholded to show voxels significant at $p<0.05$.

Pain ratings reported after the scan also did not correlate significantly with the perfusion in a one-sample t-test with age, sex and the mean grey matter perfusion as covariates of no interest, neither during the arm-up condition nor arm-down condition.

Perfusion differences between patients and healthy controls

A between-group analysis of the perfusion images acquired during arm-down condition, i.e., a two sample t-test between patients and controls with age, sex and the mean grey matter perfusion as covariates of no interest, did not result in significant differences. The same analysis of the arm-up condition showed increased perfusion in patients in a cluster encompassing the thalamus, midbrain, and the cerebellum; with additional clusters in the temporal lobes and orbitofrontal cortex (Fig. 7 and Table 3). There were no significant results for the opposite contrast (controls>patients).

Fig 7 Between group differences for the arm-up condition between patients and controls.



Two-sample t-test in 55 patients and 20 control subjects during the arm-up condition (patients > controls contrast, with age and sex as covariates of no interest). Randomise run with n=5000 permutations within grey matter mask. The statistical image was FWE-corrected using threshold-free cluster enhancement (TFCE). The results were thresholded to show voxels significant at $p<0.05$.

Table 3 Maximum p-values within each cluster for the comparison presented in Fig. 7.

Voxels	p-max	X (mm)	Y (mm)	Z (mm)	Location
8718	1	30	-42	-38	cerebellum
2039	0.999	54	-26	-30	right inferior temporal gyrus
1291	0.99	-44	-26	-26	left inferior temporal gyrus
638	0.983	18	26	-18	right orbitofrontal cortex
60	0.96	-12	26	-20	left frontoorbital cortex
7	0.952	-30	46	-12	left frontal pole
6	0.952	-46	-78	-12	left lateral occipital cortex
5	0.961	-30	-68	-10	left fusiform gyrus
3	0.952	-36	-14	-14	left hippocampus

Post hoc ROI analysis

We performed post hoc analysis within three ROI identified in the within-group analysis on data flipped along the x-axis for the patients with affected left arm (i.e., results presented in Fig. 5). As the largest cluster with the peak in the cerebellum extended from the thalamus, through the midbrain, we have selected a peak activation within the thalamus only (using a mask based on the Harvard-Oxford Subcortical Atlas thresholded at 50%) because pain-related changes in the thalamus were reported in earlier PET studies.[18] We have extracted mean perfusion values

from three spherical ROIs with 5mm radius and peak in the left primary somatosensory cortex (coordinates in mm: -26, -26, 56), the left operculum (coordinates in mm: -50, 4, 8) and the left thalamus (coordinates in mm: -10, -8, 6).

A regression analysis of mean perfusion values within each ROI adjusted for group, condition, age, sex, and mean perfusion value, clustered by subject, was run for each ROI (Appendix 2, Supplementary Table 1). For the left primary sensory cortex ROI there was a significant increase in perfusion for arm-up condition (β=3.86 ml/100g/min, 95%CI 2.42 to 5.30) but not for group (β=1.45 ml/100g/min, 95%CI -3.49 to 6.38). A similar effect was present in the left operculum, with an effect for condition (β=3.30 ml/100g/min, 95%CI 1.84 to 4.77) but not for group (β=2.22 ml/100g/min, 95% CI -0.66 to 5.10). Whereas, for thalamus the effect of condition (β=3.00 ml/100g/min, 95%CI 1.85 to 4.10) and group (β=4.17 ml/100g/min, 95%CI 0.75 to 7.58) were significant. In all three analyses the effect of the mean global perfusion was highly significant ($p<0.0005$).

The paired t-test of the ROI perfusion in the left primary somatosensory cortex between the arm-up and arm-down condition in the patient group was statistically significant (t=-3.38, p=0.0014) and the change in perfusion was from 41.38ml/100g/min (SD 12.19) to 44.68ml/100g/min (SD 14.51) so the difference was 8%; for the ROI in the left operculum the perfusion change was from 61.58 ml/100g/min (SD 9.56) to 64.56 ml/100g/min (SD 11.58) (t=2.87 p=0.0059) which is 5%, and for the ROI in the thalamus the difference was from 53.30 ml/100g/min (SD 10.08) to 55.10 ml/100g/min (SD 11.28) (t=1.99 p=0.052) which is 3%.

We also investigated whether, in the patient group, pain ratings explained the perfusion changes in these ROIs (i.e., regions which showed significant differences between arm-up and arm-down condition) using a regression model with ROI

perfusion, pain ratings, the mean grey matter perfusion, condition, age, sex and clustering by subject (Appendix 2, Supplementary Table 2). For the left primary somatosensory cortex, the effect of condition was significant (β=6.02 ml/100g/min, 95%CI 2.03 to 10.016) but the effect of pain rating was not (β=-0.29 ml/100g/min, 95%CI -1.19 to 0.60). For the ROIs in the left operculum and the left thalamus neither effect was significant when pain ratings were entered into the model (operculum: condition β=3.18 ml/100g/min, 95%CI -0.69 to 7.05, pain ratings β= 0.19 ml/100g/min, 95%CI -0.50 to 0.88; thalamus: condition β=2.44 ml/100g/min, 95%CI -0.77 to 5.65, pain rating β=0.11 ml/100g/min, 95%CI -0.54 to 0.76).

When the condition variable was removed from the model, (Appendix 2, Supplementary Table 3) the perfusion correlated with pain ratings in the operculum (β=0.53 ml/100g/min, 95%CI 0.15 to 0.92) but not in the thalamus (β=0.37 ml/100g/min, 95%CI -0.0074 to 0.75) and the primary somatosensory cortex ROI (β=0.35 ml/100g/min, 95%CI -0.22 to 0.93).

The arm-up condition was significantly associated with higher pain ratings (β=4.38, t=13.27, 95%CI 3.72 to 5.04); therefore, we were faced with a problem of multicolinearity. In this study, the effect on arm-down reflecting pain at rest and arm-up reflecting ongoing evoked pain cannot be distinguished from changes in pain rating.

When only arm-up data in the patient group were analysed in a regression with age, sex, and the mean grey matter perfusion entered in the model (Appendix 2, Supplementary Table 4) the significant effect was present only for the mean grey matter perfusion.

DISCUSSION

Main findings

As expected, raising of the affected arm, resulted in increased pain ratings in the patient group but not in the control group. It was also associated with an increased perfusion arm-up versus arm-down in the patient group (within-group) but not in the control group and a larger perfusion in the patient group than in control group during the arm-up but not during the arm-down condition (between groups). As raising an arm did not result in a significant change in perfusion in healthy controls this suggests that the observed effect is related to raising the affected arm into a painful position not just arm repositioning.

Contrary to our expectations, no regions in voxel-wise analysis correlated with pain ratings reported by patients after the scan; however, the F-test for the condition and pain ratings explanatory variables was significant. The post hoc analysis suggested that the reason why we did not observe correlation with pain ratings was that the local perfusion changes being largely explained by the global perfusion changes and by multicolinearity in the within-group model. When the condition variable was removed from the model, there was an association between pain ratings and perfusion in the contralateral operculum, with a trend ($p=0.054$) for the thalamus.

The optimised analysis pipeline resulted in a robust registration of individual perfusion images to the group template, which was superior to standard registration.

Strengths and limitations

This study has several strengths. Firstly, it involved a clinically-meaningful stimulus, which allows us to investigate perfusion related to ongoing musculoskeletal pain that

patients experience as a result of their condition. Secondly, it used a whole-brain analysis rather than multiple pre-specified ROIs. Thirdly, it had the advantage of using both within- and between-subject analyses, which helped with the interpretation of the observed results. Moreover, the sample size in this study was two to three times larger than the previous ASL patient studies [6,3,19]. Finally, we used an optimised registration between the ASL and the structural space.

Despite being larger than the other ASL patient studies, it is possible that our study did not have sufficient power to detect subtle changes in a patient population of elderly patients. The within-group differences in signal change were around 3-8%, which may explain why we did not observe differences between patients and controls during the arm-down condition or correlations between perfusion and pain ratings.
A recent paper suggested that at least 37 older participants, mean age 74 (SD 8), per group are required to detect a 10% perfusion difference using permutation-based algorithms [20] and an earlier healthy-control study [21] demonstrated that 10 to 15 healthy controls are required to observe 15% changes in a within-subject model. Therefore, despite our sample size being large it was still probably too small to detect some of the effect as the effect was rather small. Data from older subjects, especially patients, would require a larger sample due to variance in the perfusion data related to physiological factors and due to clinical heterogeneity.

As about half of the patients reported no pain during the arm-down condition and, in general, this condition was less painful this created a problem of multicolinearity, as most of the within-group differences in pain ratings were accounted for by differences between the arm-up and arm-down condition, leaving very little variance to be explained by the actual pain ratings. This problem could not be solved with the existing data. We would either need more data (i.e., a large sample size) or more uncorrelated data (i.e., more patients reporting pain during the arm down condition)

in order to differentiate the effect of change of condition from change in pain ratings. Correlating perfusion with pain ratings during arm-up and arm-down separately was challenging because during the arm-down condition about half of the patients reported no pain and during the arm-up condition the evoked pain was likely to be affected by the pain at rest which may explain why we did not see correlations between perfusion and pain ratings during each of those scans separately. Also, unlike other studies [5,7] we did not ask patients to appraise and rate pain during the scan, but asked for a single rating after the scan. This has a disadvantage of resulting in only one rating per scan which does not allow to correlate changes in pain rating with changes in perfusion during the scan. However, it means that in our study perfusion was not confounded by pain appraisal and rating.

Finally, the order of arm-down and arm-up scans could not be randomised in case the evoked pain persisted beyond the duration of the scan, thus order-effect may be a confounder. However, we would not expect a substantial carry-over effect from lying in the scanner with arms beside the body. It is more likely that patients were more restless during the more painful and more uncomfortable arm-up scan, especially as it was performed at the end of a long scanning session. This effect might have been exacerbated in patients who experienced pain during the arm-down condition.

Interpretation

Differences between the arm-up and arm-down conditions in the patient group were observed in the structures that encode the sensory-discriminatory dimension of pain, including pain intensity[22–25]. Increase in perfusion in primary and secondary somatosensory cortex, mid-insula, amygdala, hippocampus, and midbrain was reported in an ASL study on osteoarthritis[19] In another ASL study on pain in

osteoarthritis perfusion in the ipsilateral insular cortex and operculum, putamen, the amygdala/hippocampus region, and brainstem correlated with reported pain intensity [7]. Increased activity in the midbrain, thalamus, cerebellum, primary somatosensory cortex, anterior cingulate, and insula has been also described during capsaicin induced sensitisation in healthy volunteers [26]. Therefore, the observed changes may reflect altered pain processing related to central sensitisation.

Differences between patients and controls during the arm-up condition were present mainly in the thalamus, which is the main relay centre for the pathways transmitting nociceptive information, and has been reported to be involved in chronic pain processing [18,24,27]. There was also a cluster in the midbrain, which may be related to modulation of thalamic transmission [28]. Higher levels of BOLD activation in patients than controls in the brainstem, the thalamus and cerebellum have previously been described in response to pressure pain in fibromyalgia [29]. A recent ASL study in fibromyalgia patients reported that perfusion in the putamen correlated negatively with pain disability and positively with the overall disease impact scores, whereas pain intensity correlated with perfusion in the cerebellum.[30] The authors interpreted these changes as an effect of reduced physical activity in fibromyalgia patients; although, activation in the putamen may also reflect processing of the sensory aspect of pain.[25] It is likely that the observed changes between arm-up and arm-down condition reflect maladaptive changes in the network integrating movement and sensory information as we found perfusion changes in a cluster encompassing the thalamus and the putamen as well as changes in the primary somatosensory as well as primary motor cortex.[30–32]

There were no differences between patients and controls during the arm down condition, which could have been caused by the fact that only half of the patients reported pain during the arm down condition or because the differences in perfusion

between patients and controls at rest were small while the physiological variability was large. Cottam and colleagues[7] did not show differences between patients and controls despite all their patients reporting pain on that day. In our study, the regional perfusion changes were strongly associated with changes in the mean grey matter perfusion and the variation in global perfusion across sessions and subjects was quite large, which added considerably to the variability of the absolute perfusion values.

Neither between-group or within-group analysis resulted in extensive changes in regions involved in the cognitive and emotional assessment of pain, which have been implied to be more often engaged in chronic pain than in acute pain [9]. A widespread network of prefrontal, parietal regions as well as both primary and secondary somatosensory cortices were reported in response to evoked ongoing clinical pain in lower back pain patients.[6] These changes were interpreted by the authors as reflecting affective pain processing and changes in the attention network. We also did not observe significant activation in the cingulate cortex (neither did Wasan et al.); although, along with activation in the putamen and bilateral activation in the amygdala and hippocampus, these changes have been reported to correlate with pain ratings in patients with osteoarthritis [7].

Like in Wasan et al., we did not observe significant linear correlation between changes in pain ratings and changes in perfusion. They interpreted this as an indicator that either the changes in pain ratings were too small (they expected to see change in perfusion related to pain only when pain ratings increased > 30%) or that perfusion change was not a marker of pain severity [6]. However, it is also possible that there is so much variability in perfusion data that it is difficult to detect finer changes. In our study, the lack of significant correlations between changes in pain ratings and perfusion during the arm-up and arm-down condition was most likely

caused by multicolinearity as most of the difference between pain ratings during the arm-up and arm-down condition was accounted for by the difference between the conditions, leaving very little variance that could be explained by the covariate representing change in the reported pain ratings.

CONCLUSIONS

Arm repositioning may be used as a paradigm for evoked ongoing musculoskeletal pain in patients with shoulder impingement. It results in perfusion changes related to pain rather than change of position as the changes were not observed in the control group. By optimising the analysis pipeline, it is possible to study perfusion related to ongoing clinical pain processing. Using ASL to study clinical pain has the potential to assess effectiveness of existing treatments and to develop new therapies for chronic pain.

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