

Quantitative Changes in Regional Cerebral Blood Flow Induced by Cold, Heat and Ischemic Pain

A Continuous Arterial Spin Labeling Study

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ABSTRACT

Background: The development of arterial spin labeling methods has allowed measuring regional cerebral blood flow (rCBF) quantitatively and to show the pattern of cerebral activity associated with any state such as a sustained pain state or changes due to a neurotropic drug.

Methods: The authors studied the differential effects of three pain conditions in 10 healthy subjects on a 3 Tesla scanner during resting baseline, heat, cold, and ischemic pain using continuous arterial spin labeling.

Results: Cold pain showed the greatest absolute rCBF increases in left anterior cingulate cortex, left amygdala, left angular gyrus, and Brodmann area 6, and a significant rCBF decrease in the cerebellum. Changes in rCBF were characteristic of the type of pain condition: cold and heat pain showed increases, whereas the ischemic condition showed a reduction in mean absolute gray matter flow compared with rest. An association of subjects' pain tolerance and cerebral blood flow was noted.

Conclusions: The observation that quantitative rCBF changes are characteristic of the pain task used and that there is a consistent rCBF change in Brodmann area 6, an area responsible for the integration of a motor response to pain, should provide extremely useful information in the quest to

What We Know about This Topic

- The pattern of cerebral activity associated with states such as a sustained pain state or changes due to a neurotropic drug are not well characterized

What This Article Tells Us That Is New

- Using continuous arterial spin labeling, regional cerebral blood flow changes were characteristic of a pain task employed and may provide evidence for an imaging biomarker of pain

develop an imaging biomarker of pain. Conceivably, response in BA6 may serve as an objective measure of analgesic efficacy.

IN recent years magnetic resonance imaging (MRI)-based brain mapping techniques have significantly enhanced the ability of neuroscientists to associate brain anatomy with function. Most functional imaging work is based on the blood oxygen level dependent (BOLD) susceptibility difference of oxyhemoglobin and deoxyhemoglobin,^{1,2} which essentially reflects capillary vasodilatation in response to regional neuronal activity in the brain. Blood oxygen dependent level functional magnetic resonance imaging (BOLD fMRI) depends on changes in activity between conditions and therefore cannot directly assess the regional cerebral blood flow (rCBF) associated with a single state (for example, rCBF at rest or rCBF in a drug state). Because of the limitations of BOLD fMRI, we have previously used H₂¹⁵O based positron emission tomography to study the effect of propofol, a commonly used anesthetic drug, on brain areas functionally associated with wakefulness and the processing of pain.³ This diffusible tracer-based perfusion technique requires repeated arterial blood sampling, the availability of a cyclotron to produce the radiotracer and the number of scans are limited by the safe maximum dose of the radiotracer, H₂¹⁵O.

Noninvasive alternatives to positron emission tomography are arterial spin labeling (ASL) MRI methods. ASL is accomplished by inverting spins upstream of the imaging slice at which perfusion is to be measured,^{4,5} so that the inverted magnetization of the blood water acts as a tracer. With pulsed arterial spin labeling techniques, a volume of blood is labeled upstream of the region of interest by a short

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radiofrequency pulse. With continuous arterial spin labeling techniques (CASL), an inversion pulse is applied continuously in the direction of flow. In addition to quantifying rCBF increases, this method allows us to examine whether certain pain tasks induce a reduction in rCBF, a possibility that is being overlooked by many BOLD fMRI based studies.⁶ However, some early positron emission tomography reports indicated that task related blood flow reductions do occur in certain pain states.^{7–9}

We tested the hypothesis that cold, heat, and ischemic pain induce the different rCBF changes within regions considered part of the “pain matrix”¹⁰ using CASL fMRI. Instead of using very brief pain pulses characteristic for BOLD fMRI studies, we used sustained stimuli that would be perceived as moderately to severely painful without incurring the risk of tissue damage (cold and ischemic pain). Sustained tasks such as ischemic pain and cold pain are thought to represent clinical pain better due to their psychophysical qualities^{11–13} and are predictive of clinically relevant doses of analgesics^{14–16} as well as acute and chronic pain-related clinical outcomes.^{17,18} We expected the side-by-side comparison of the pain tasks to reveal characteristic differences in brain activation. Finally, we examined some potential relationships between rCBF and individual pain tolerance levels.

Capturing data to evaluate pain type specific brain activation would help us to examine the utility of pain imaging as a marker for analgesic treatments. Information on the correlation of pain tolerance and cerebral blood flow (CBF) would indicate whether subjective experience of pain reflects an individual’s task induced CBF.

Materials and Methods

Subjects

The Institutional Review Board of the University of Alabama at Birmingham approved this study. Recruitment was performed by public advertisement around the university campus. Interested individuals were scheduled for a screening visit during which we determined eligibility by obtaining a medical history. We performed a focused physical examination and obtained written informed consent. Enrollment started in April 2009 and finished on January 2012. Inclusion criteria were right-handed healthy adults, age 19–50 yr, who were able to understand all study instructions. Handedness was assessed using the Edinburgh Handedness Inventory.¹⁹ Exclusion criteria were any existing and active medical conditions that could affect somatosensation or cognitive function such as diabetes mellitus, neurologic diseases, chronic pain, psychiatric disorders, the treatment with any scheduled medication, and a past history of drug or substance abuse. Volunteers were paid for their participation and scheduled for a visit to obtain psychophysical measures and functional imaging on separate days.

Pain thresholds were obtained for each participant using a small 3 × 3-inch thermal probe (Thermal Sensory Analyzer TSA-II, Medoc Ltd., Ramat Yishai, Israel) applied to the skin

of the forearm. Detection of the pain tolerance were assessed on the ventral forearm using an ascending method of limits with a 1°C/s rate of rise (heat) whereby participants felt the temperature rise of the heat probe and were instructed to terminate the task by clicking a mouse button whenever they were no longer able to tolerate the thermal stimulus.

Experimental Pain Tasks

Subjects underwent CASL imaging at rest and while experiencing three pain conditions. The order of study acquisitions for all participants was: Survey scan (1 min) for slice orientation, CASL rest (no pain stimulus), CASL heat, CASL ischemic, CASL cold pain, and anatomical (T1-weighted) image. The rest scan was approximately 8 min for the acquisition of 42 tagged and untagged images, each pain session was approximately 5.5 min long for the acquisition of 30 tagged and untagged images and anatomical scans were approximately 1 (survey scan) and 6 min in duration. There was an approximately 5-min wait period between imaging sessions to advance the scanner settings and to adapt the stimulation setup. Participants remained in the scanner during setup and these steps for a total time of approximately 60 min.

To induce sustained but tolerable cold pain, we alternated the immersion of the volunteer’s right hand into plastic cups filled with either ice water (2–3°C) or tepid water (30°C) for 10 s at a time during the cold pain imaging session. Heat pain was delivered using an MRI compatible, size 3 × 3-inch thermal probe (Medoc Ltd.) which was firmly applied to the right forearm of the participant. Each heat block consisted of an 8-s thermal stimulus that was programmed to fluctuate in a sinusoidal pattern within 46–49.5°C. Heat alternated with an 8-s block of neutral (32°C) temperature. The reason for alternating blocks of pain with a neutral stimulus in the cold and heat pain conditions was to avoid habituation. If we had applied a constant thermal (cold or heat) stimulus, the intensity would have had to be relatively low to make it tolerable for participants.

Unlike the thermal tasks, the ischemic task was maintained continuously for the 5-min duration of the scan session. This standardized task had the participant increase the right arm for 30 s, after which a cuff covering the upper arm was inflated to 250 mmHg. The participant was then instructed to perform handgrip exercises for 1 min. Upon completion of the handgrip exercises, the imaging session was started.

Imaging Parameters and Preprocessing

High-resolution structural MRI and quantitative ASL perfusion MRI were obtained at the University of Alabama cardiovascular imaging facility using a 3 Tesla magnetic resonance Phillips Achieva scanner (Phillips Healthcare, Andover, MA). Structural imaging was performed with a T1-weighted three-dimensional MPRAGE sequence with TR/TI/TE = 1,620/950/3 ms, flip angle = 30°, matrix = 192 ×

256 × 160, and voxel size = 0.98 × 0.98 × 1 mm³. ASL images were acquired using a CASL sequence with the following parameters: 1,600 Hanning window shaped pulses for a total labeling time = 2.4 s, postlabeling delay = 1.4 s, FOV = 230 mm, matrix = 128 × 128, bandwidth = 3 kHz/pixel, TR/TE = 5000/56 ms, 13 slices with thickness of 7 mm plus 1.25 mm gap, mean Gz of 0.6 mT/m, labeling was placed at 80 mm below the center of imaging region. There were 42 tag/control image pairs that were acquired during the resting state, and 30 tag/control image pairs were acquired at each pain stimulation condition. Respiratory rate (RR) and end-tidal carbon dioxide values were recorded during each scan by sampling from a nasal cannula connected to a Capnomac Ultima carbon dioxide and anesthesia analyzer (General Electrics Healthcare, Waukesha, WI).

ASLtbx,²⁰ an ASL data processing toolbox based on Matlab (Mathworks, Natick, MA) and SPM (Wellcome Department of Cognitive Neurology, London, United Kingdom), were used for imaging analysis. The following preprocessing steps were used: motion correction, residual motion effect removing, denoising, registration, spatial smoothing, CBF calculation, and CBF map spatial normalization. Both the labeled and control images were realigned together using the six parameters-based rigid body transformation to minimize the distance between each image and the reference volume. The algorithm was implemented in SPM. Then, the zigzagged labeled/control spin labeling paradigm was used as regressor in the estimated motion time courses, and the cleaned motion time courses were finally used for real motion corrections. No low-pass filtering was applied for ASL data. Spatial-temporal noise reduction was then performed using principal component analysis.²¹ The nonzero eigenvalue associated eigenvectors and their time-courses were extracted by applying principal component analysis along the spatial dimension of ASL data to avoid the computational problem of applying principal component analysis along the temporal dimension. Each image was projected into these eigenvectors to obtain the corresponding representation coefficients. The following criteria were used to discard the noise-related eigenvectors and the corresponding representing coefficients to further denoise the raw ASL data: (1) any eigenvectors whose time-courses were correlated with the zigzagged label-control paradigm were reserved; (2) minor components of the remaining eigenvectors were discarded if they accounted for less than 1% of the total variance of the data before principal component analysis denoising.

Calculation of rCBF

The motion-corrected ASL scans were then compartmentalized into groups of control or labeled images. Signal changes between the control and labeled images are much lower compared with signal changes observed in BOLD imaging. It is thus imperative to obtain rCBF maps before transforming the images to the Montreal Neurology Institute template. The variation of signal intensities within control and labeled

set of images made it necessary to average signal values for all images within each compartment before we calculated the CBF values. Quantitative CBF maps were then calculated from the control-tag perfusion signal difference using a modified two-compartment ASL perfusion model²²:

$$f = \frac{\Delta M \cdot \lambda \cdot R_{1\alpha} \cdot \exp(\omega \cdot R_{1\alpha})}{2 \cdot M_0 \alpha} [1 - \exp(-t \cdot R_{1\alpha})]^{-1} \quad (\text{Equation 1})$$

where f is CBF, ΔM is the difference signal between the control and label acquisitions, $R_{1\alpha}$ is the longitudinal relaxation rate of blood, τ is the labeling time, ω is the post labeling delay time, α is the labeling efficiency, λ is blood/tissue water partition coefficient, and M_0 is approximated by the control image intensity. The parameters used in this study were $R_{1\alpha} = 1/1,664$ s, $\alpha = 0.85$, $\lambda = 0.9$ g/ml, $\tau = 2.4$ s, $\omega = 1.4$ s. The CBF maps so generated were then normalized to the Montreal Neurology Institute template and smoothed using a 8 mm × 8 mm × 8 mm Gaussian kernel.

Selection of Regions of Interest

Based on the existing literature on pain imaging we selected the following regions of interest (ROIs): thalamus, precentral and postcentral cortex, insular cortex, cingulate gyrus, anterior and posterior cingulum, amygdala, hippocampus, and Brodmann areas 1 to 4 and 6.²³ These ROIs were selected with the Wake Forest University (Winston-Salem, North Carolina) pick atlas, which uses the Talairach Daemon database.²⁴ From within these ROIs, we extracted the voxel signal intensities of labeled and unlabeled images and calculated rCBF as described in equation 1.

Statistical Analysis Steps

We used the statistical software SAS (SAS Institute, Cary, NC) version 9.2, procedures GLM, MIXED, TTEST, and UNIVARIATE, for our statistical analysis.

Analysis 1: Preliminary Analyses

As preliminary, diagnostic analysis steps we compared global cerebral blood flow (blood flow of all voxels within the brain), RR and end-tidal carbon dioxide to ensure that these variables did not change significantly when comparing conditions (baseline and pain conditions) in separate analyses. The model for the global blood flow analysis was the following general linear model:

$$CBF = \alpha + \beta_1 * ID + \beta_2 * PC + \varepsilon, \quad (\text{model 1})$$

where CBF represents global cerebral blood flow, α represents the intercept, β_1 represents the parameter vector associated with the subject effect (ID) and β_2 represents the parameter vector associated with the three pain conditions. In this analysis, we did not consider subject as random effects

because we were interested in obtaining a parameter estimate for the subject effect to inform us about the differences of global blood flow across participants.

Although the latter analyses used blood flow data from all voxels within the brain, subsequent analyses were restricted to voxels within the regions of interest. RR and end-tidal carbon dioxide were compared with resting baseline levels for each across pain conditions using paired Student *t* tests. We considered a Bonferroni adjustment of the probability for rejecting the statistical null hypothesis ($\alpha = 0.05/3$) for the latter analysis.

Analysis 2: Comparison of Pain Conditions

We then directed our attention to our ROIs, gray matter regions functionally associated with pain. To better select voxels associated with gray matter we adopted the approach of selecting voxels with higher rCBF values. The method of selecting voxels with higher flow values has been used routinely in the analysis of single photon-emission computed tomography data and is one of several approaches to avoid the inclusion of voxels that contain a mixture of multiple tissue values, also known as partial volume effect.^{25,26} We chose the top 50% selection criteria for subsequent statistical analyses. We compared CBF change (baseline *vs.* pain) for each of the three pain conditions. In this comparison we controlled for the subject-specific (CBF) effects by entering subjects as random variable. We used the following mixed model:

$$\Delta rCBF = \alpha + id + \beta * PC + \varepsilon, \quad (\text{model 2})$$

where *id* represents the subject random effect and *PC* represents pain condition vector, the explanatory variable of interest. Epsilon (ε) represents the residual error. We planned to compare pain conditions with Student *t* tests as a follow-up procedure if the overall model showed significant differences.

Analysis 3: Blood Flow Changes across ROIs

To quantify rCBF changes across ROIs, we used a mixed model where subjects were entered as random variable, pain condition was nested within the subject effect, and regions of interest were entered as fixed effect:

$$\Delta rCBF = \alpha + id[PC] + \beta_1 * PC(ROI) + \beta_2 * ROI + \varepsilon, \quad (\text{model 3})$$

where *id[PC]* represents the subject random variable nested within pain conditions (*PC*) with associated parameter vector β_1 , *PC(ROI)* represents the pain condition nested within *ROI* with associated parameter vector β_2 , and epsilon (ε) represents the residual error.

Analysis 4: Correlation of Pain Tolerance and Cerebral Blood Flow

We then determined whether participant's pain tolerance was correlated with CBF using a bivariate regression with

heat pain tolerance as explanatory variable and mean rCBF, averaged across ROIs, as outcome variable:

$$rCBF = \alpha + \beta * HPT_0 + \varepsilon, \quad (\text{model 4})$$

where α represents the intercept, β represents the parameter vector for each participants' heat pain tolerance (*HPT₀*) and ε represented the residual error. We performed separate analyses for each of the three pain conditions and considered a Bonferroni adjustment of the probability for rejecting the statistical null hypothesis ($\alpha = 0.05/3$). Heat pain tolerance was used as tolerance measure because it is a widely published and reliable method.

Analysis 5: SPM Analysis without Restriction to ROIs

Finally, we determine whether a similar pattern of brain activity would be observed if the analysis was carried out without restriction to regions of interest. After data preprocessing as described above, this analysis was carried out using the general linear model²⁷ built into statistical parametric mapping (SPM version 8). A general linear mixed model was used to generate a statistical probability map for the group analysis.²⁸ The latter is referred to in SPM as second-level analysis.

Results

Participants consisted of five men and five women. The median age was 31 yr with a range from 19 to 49 yr. Six participants were African American and four participants were Caucasian.

Results of Analysis 1 (Global CBF)

We observed that global CBF differed across participants ($F = 73.40$, $P < 0.001$). When accounting for subject-specific global cerebral blood flow, global CBF values did not differ statistically ($P = 0.2406$) across conditions. We illustrate the effect of selecting the top 20% and 50% flow values in figure 1. This figure is also suitable to contrast and compare rCBF and the shape of rCBF distributions.

Average participants' RR at rest was 15.6 ± 3.8 and the end-tidal carbon dioxide level at rest was 38.6 ± 2.4 . During the cold condition average participants' RR was 16.3 ± 3.2 and end-tidal carbon dioxide level was 38.5 ± 3.3 . During the heat condition average patients' RR was 15.9 ± 3.5 and end-tidal carbon dioxide level was 39.0 ± 3.2 . During the ischemic condition RR was $RR = 16.1 \pm 4.0$ and end-tidal carbon dioxide level was 39.1 ± 3.3 . Respiratory rate and end-tidal carbon dioxide levels during each pain condition were not statistically different from their resting baseline values: $P = 0.9391$ (t-ratio = 0.0775) for rest *versus* cold, $P = 0.755$ (t-ratio = 0.3162) for rest *versus* heat, and $P = 0.7029$ (t-ratio = 0.3875) for rest *versus* ischemia.

Results of Analysis 2 (Comparison of Pain Conditions)

Figure 1 shows the distribution of rCBF values within our ROIs as boxplots and histograms by condition. It can be seen that blood flow values at rest cover a relatively large range of

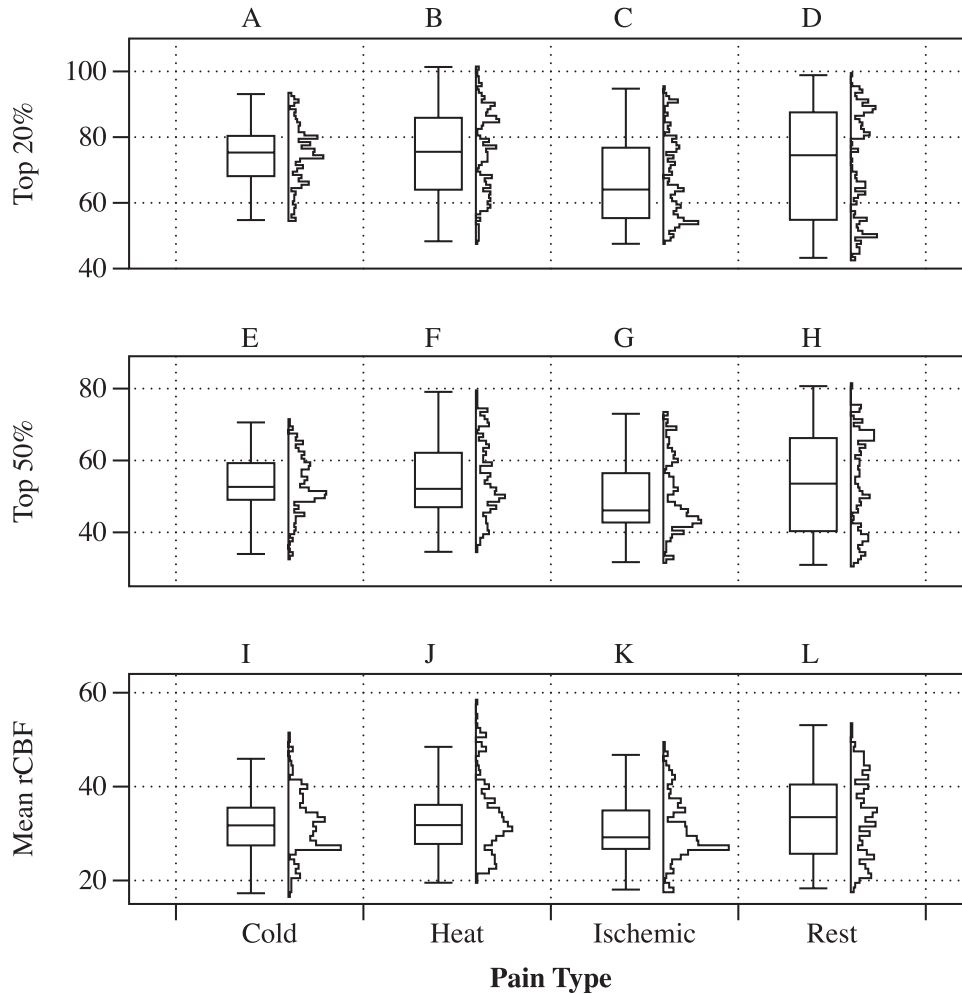


Fig. 1. The figure shows corresponding boxplots and vertical histograms of rCBF values calculated separately for each region of interest and each participant. (A–D) Summary plots for the top 20% blood flow data. (E–H) Summary plots for the top 50% blood flow data. (I–L) Summary plots for the top mean (average) blood flow data. The categorical axis (x-axis) shows experimental conditions. Regions of interest included were BA 1–4, BA 6 insular and cingulate cortex, amygdala, hippocampus, thalamus, and cerebellum. BA = Brodmann area; rCBF = regional cerebral blood flow.

values. During the cold pain condition, blood flow values tend to have a more unimodal (centered) distribution. Compared with the resting condition, a change in the shape of the histogram can also be seen for heat and ischemic pain. When comparing rCBF values across ROIs in the mixed model analysis, we observed a significant difference in rCBF change (pain *vs.* rest, $P < 0.0001$). Regional CBF within the pain matrix (averaged across pain ROIs) showed a small increase for the cold pain task [Δ rCBF = 0.35 ml/100 g/min] and heat pain [Δ rCBF = 1.00 ml/100 g/min] but ischemic pain showed a significant rCBF reduction [Δ rCBF = -3.70 ml/100 g/min].

Analysis 3: Blood Flow Changes across ROIs

We found a significant difference in rCBF change (Δ rCBF) when comparing ROIs. These are listed in table 1 and changes are illustrated in figure 2. By designating “subject” nested within “pain condition” as random effect in this mixed model, we accounted for the randomness of rCBF due

to participants’ biologic differences. The overall model fit was very good (adjusted $R^2 = 0.893$, $P < 0.0001$). We observed that the pain condition effect, nested within ROI, was not significant (F ratio = 0.960, $P = 0.561$) but the ROI effect was (F ratio = 1.740, $P = 0.0105$). This indicated that observed change in regional cerebral blood flow (Δ rCBF) depended on the anatomical location within the pain matrix, which we refer to as ROI. To determine which of the anatomical brain ROIs showed the most change, we evaluated the parameter estimates for each region of interest and observed a 2.491 ml/100 g/min rCBF decreases in both cerebellar hemispheres (t-ratio = -3.09, $P = 0.0024$), a 2.291 ml/100 g/min rCBF increase to the anterior cingulate gyrus (t-ratio = 3.28, $P = 0.0011$), a 1.853 ml/100 g/min increase rCBF increase to the left amygdala (t-ratio = 2.93, $P = 0.0035$), and a 1.670 ml/(100 mg*min) rCBF increase in BA 6 (t-ratio = 2.238, $P = 0.0238$).

Figure 3 shows CASL rCBF images of a case example as well as mean group rCBF images for the resting state and cold

Table 1. rCBF Change in ROIs

ROI	Δ rCBF	t-Ratio	P Value (> t)	95% CI	
				Lower	Upper
lacc	2.291	2.8	0.005	0.684	3.897
lamyg	1.853	2.26	0.024	0.246	3.459
lba1	0.366	0.45	0.655	-1.241	1.972
lba2	0.387	0.47	0.637	-1.220	1.993
lba3	0.785	0.96	0.338	-0.822	2.391
lba4	0.774	0.95	0.345	-0.833	2.380
lba6	0.688	0.84	0.400	-0.918	2.295
lcb1m	-2.491	-3.05	0.002	-4.098	-0.885
lcing	0.594	0.33	0.745	-2.984	4.172
lhipp	-0.312	-0.38	0.703	-1.919	1.294
lins	0.375	0.46	0.647	-1.231	1.982
lthal	-0.008	-0.01	0.992	-1.614	1.599
racc	-0.189	-0.23	0.817	-1.796	1.417
ramyg	-0.410	-0.5	0.617	-2.016	1.197
rba1	-0.872	-1.07	0.287	-2.478	0.735
rba2	-0.858	-1.05	0.295	-2.464	0.749
rba3	-0.241	-0.3	0.768	-1.848	1.365
rba4	-0.269	-0.33	0.743	-1.875	1.338
rba6	1.700	2.08	0.038	0.093	3.306
rcb1m	-1.848	-2.26	0.024	-3.455	-0.242
rcing	-0.682	-0.83	0.405	-2.289	0.924
rhipp	-0.941	-1.15	0.251	-2.547	0.666
rins	-0.241	-0.29	0.769	-1.847	1.366

lacc = left anterior cingulate cortex; lamyg = left amygdala; lba1-4,6 = left Brodmann area 1-4,6; lcb1m = left cerebellum; lcing = left cingulate gyrus; lhipp = left hippocampus; lins = left insula; lthal = left thalamus; racc = right anterior cingulate cortex; ramyg = right amygdala; rba1-4,6 = right Brodmann area 1-4,6; rCBF = regional cerebral blood flow; rcb1m = right cerebellum; rcing = right cingulate gyrus; rhipp = right hippocampus; rins = right insula; ROI = region of interest; rthal = right thalamus.

pain scans. It illustrates how rCBF increases in the ACC is evident in the single case and are also confirmed in general in the group data. The coordinates and planes shown also capture some of the marked activity increase in BA 6.

Analysis 4: Association of Pain Tolerance and rCBF

We observed a significantly higher rCBF in response to cold pain in individuals with higher pain tolerance ($P = 0.0314$). Given the Bonferroni adjustment, this difference is not statistically significant but can be described as an important trend. No association was observed for either heat or ischemic pain.

Analysis 5: SPM of CASL Images

The results of statistical parametric mapping analyses by pain task are listed in tables 2, 3 and 4 and illustrated in figure 4. These results differ somewhat from the direct analysis because in the SPM analysis we did not restrict our search to pain ROIs. We list areas of the brain that meet the uncorrected statistical threshold of $P = 0.001$. The observed changes agree with our ROI-based analysis in several key areas. With respect to the cold task, the most notable finding is the activation of brain areas functionally associated with

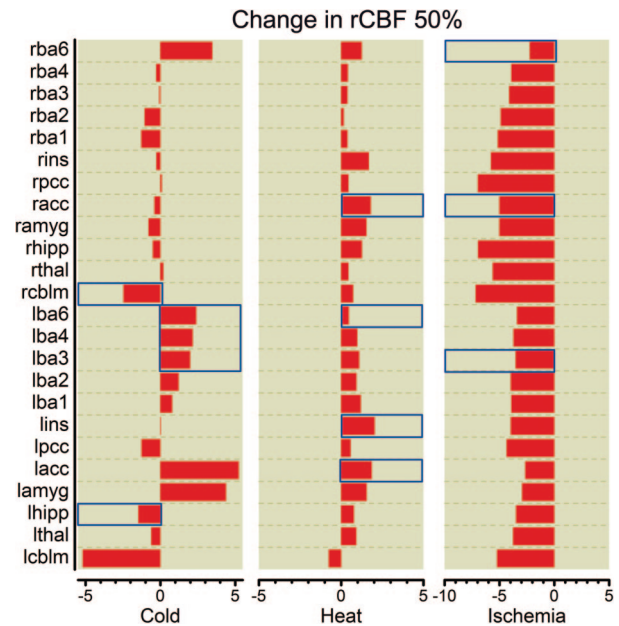


Fig. 2. Average regional cerebral blood flow (rCBF) values in regions of interest (ROIs) using the top 50% voxel selection filter. Areas that can also be identified by the statistical parametric mapping analysis that was not restricted to pain ROIs are marked with a transparent blue box.

somatosensory processing (precentral and postcentral gyrus) and integration (Brodmann area 6). We found heat pain to be associated with activation in the anterior cingulate gyrus bilaterally, left insula (Rolandic operculum) and inferioparietal cortex. We found ischemic pain to be associated with an rCBF increase in the left insula and thalamus whereas Brodmann area 6 and postcentral gyrus show deactivation (reduced rCBF) with this modality.

Discussion

The most salient findings of our study, in detail described in the following subheadings, are that (1) pain-related rCBF changes are task specific and (2) across modalities the most consistent change can be observed in the supplemental motor cortex (Brodmann area 6) rather than brain regions associated with somatosensory brain areas. We introduce CASL imaging as a method to quantify state-dependent pain measures. This is clinically relevant because, in the practice of anesthesiology, we are concerned with drug states such as the state of anxiety (before surgery), the sedated state (during a clinical procedure), or the analgesic state (of a patient having received opioid analgesics). As objective measures of changes in central nervous system activity that can be task and state specific, CASL imaging allows for the assessment of complex cognitive processes and how these activities can be altered by manipulations such as anesthesia.

Detecting Pain Task-specific rCBF Patterns

One of the most striking observations was the reduction in rCBF within the pain ROIs seen with ischemic pain, whereas

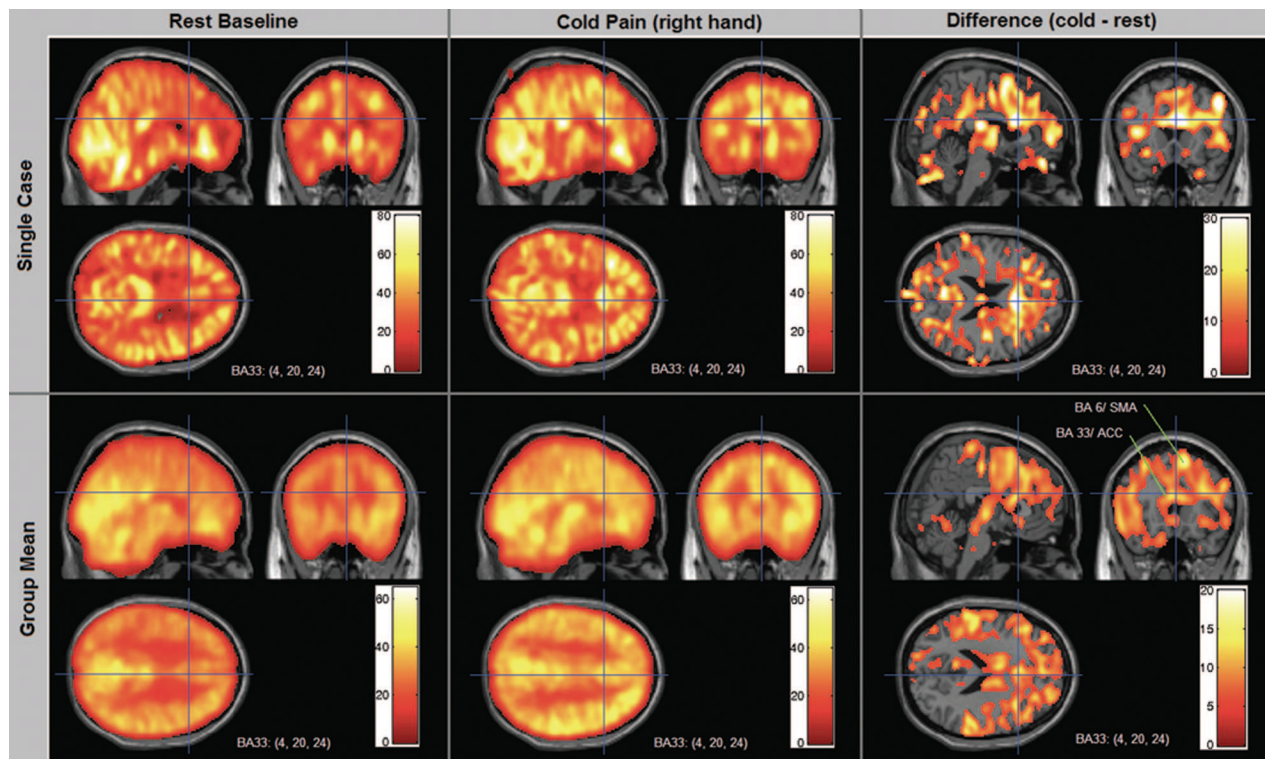


Fig. 3. Continuous arterial spin labeling (CASL) regional cerebral blood flow (rCBF) images for a single case and for the mean of all 10 cases during rest and the cold pain condition. In this example the crosshairs and corresponding orthogonal slices are centered on the anterior cingulate cortex (ACC), Montreal Neurology Institute coordinates $x = 4$, $y = 20$, $z = 24$. The group images were created from the mean of 10 motion-corrected individual case image sets, spatially normalized into stereotactic space. Color bar scales indicate rCBF in ml/100 g/min. Increased rCBF is evident in the ACC during cold pain by visual inspection of the case example (the actual rCBF increases from 49.6 at rest to 72.9 at the coordinate center voxel). The group mean images show that this increase is evident across subjects, both by visual inspection and quantitative values (group mean rCBF increases from 41.9 to 57.4 at the coordinate center voxel). The difference images on the right side were created by subtracting the resting baseline rCBF values from the cold pain rCBF values in all voxels. The ACC (Brodmann area 33) appears as the area of greatest flow increase in these coordinate planes, along with part of the supplementary motor cortex (Brodmann area 6) shown in these planes. The lower right panel identifies these two regions in the mean group subtraction.

thermal (cold and heat) pain showed no net change or a net increase in rCBF. Upon closer examination, we can appreciate that the overall pattern of the blood flow distribution is similar to the one observed in other pain modalities (fig. 2).

As described, we estimated “global CBF” by calculating the mean of all brain voxels with a nonzero rCBF. We observed a small reduction of global CBF in the cold condition when compared with the resting state whereas no change of global blood flow was observed for the ischemic condition. By contrast, within the pain ROIs, cold pain resulted in a net blood flow increase and ischemic pain resulted in a net blood flow decrease. Although most of the BOLD fMRI literature focuses on areas with relative signal increases, task-related blood flow reductions have been reported before.^{7–9} These small changes in global rCBF during the cold and ischemic task may represent minimal effects of peripheral circulation changes not completely adjusted for by global cerebral autoregulation.²⁹ This finding calls attention to the need for close examination of relative signal or rCBF decreases when examining and describing the effects of pain.

Brodmann Area 6 as Potential Biomarker of Acute Pain

We found Brodmann area 6 to receive significantly higher rCBF across pain modalities. This relatively large cortical area represents a bilaterally organized system involved in the acquisition and performance of skilled motor acts including speech and writing³⁰ and preparation for and the sensory guidance of movement.^{31–33}

An intuitive explanation for the activation of Brodmann area 6 activated across pain modalities is based on its involvement in the planning of motor responses. In our experiment participants did not move the right arm, despite its subject to the pain task, presumably suppressing the urge to withdraw the arm from the painful stimulus. The supplemental motor area (Brodmann area 6) and ventrolateral prefrontal cortex have been implicated in the suppression of a motor task.³⁴ This is consistent with a recently documented cognitive role of Brodmann area 6; Tanaka *et al.*³⁵ reported Brodmann area 6 activation in response to both verbal and spatial mental operation using BOLD fMRI and a decreased performance of the mental operation if

Table 2. Statistical Parametric Mapping Cold Pain

X	Y	Z	Cluster Size	Anatomy I	Anatomy II	t-Value
Activation						
-18	7	64	36	L Superior frontal gyrus	BA 6	7.07
-20	55	32	28	L Superior frontal gyrus	—	8.82
-48	-74	20	17	L Middle temporal gyrus	IPC (PGp)	4.81
-40	-22	57	15	L Precentral gyrus	BA 4a	4.77
—	—	—	—	—	BA 6	—
-36	-32	60	6	L Postcentral gyrus	BA 3b	5.31
—	—	—	—	—	BA 4a	—
-46	8	42	6	L Precentral gyrus	BA 44	5.09
Deactivation						
32	-18	-24	—	R Parahippocampal gyrus	Hipp (SUB)	6.59
—	—	—	—	—	Hipp (CA)	—
36	-16	-26	—	R Fusiform gyrus	Hipp (CA)	—
21	4	-24	49	R Parahippocampal gyrus	Hipp (EC)	4.3
54	-6	-27	14	R Inferior temporal gyrus	—	—

BA 3b = Brodmann area 3b; BA 4a = Brodmann area 4a; BA 6 = Brodmann area 6; BA 44 = Brodmann area 44; Hipp (CA)⁴¹ = hippocampus area CA⁴¹; Hipp (EC) = Hippocampus area EC⁴¹; Hipp (SUB) = hippocampus area SUB; IPC (PGp) = inferior parietal cortex, area PGp^{39,40}; L = left; R = right.

subjects received transcranial stimulation to Brodmann area 6. Within this context, the blood flow change we observed may be best described as an associated cognitive mental response. Though activation of the supplemental motor area has been observed in other studies, we highlight this area in our discussion because it was both significant across pain tasks and draws attention to the potential utility of monitoring the motor response to a painful stimulus as a means of assessing the efficacy of an analgesic intervention that does not rely on a patient's subjective interpretation of a painful stimulus. This finding is clinically relevant because one of the main goals of pain imaging is to find an objective measure of analgesia.

Subjective Pain Tolerance and rCBF

The notable correlation observed between individual pain tolerance measures, which reflect a person's subjective ex-

perience, and rCBF during the cold condition, without doubt an objective measure, is a very promising relationship suggesting the possibility of using certain rCBF measures as a biomarker of pain. It is interesting to note that there is some evidence to suggest that pain, when persistent, is associated with characteristic changes in rCBF.^{36–38} Although the correlation observed in these studies may not be robust enough to suggest making clinical decisions about individual cases, these data suggest that the promise of imaging biomarkers of certain perceptual states may be realizable. Issues such as drug-seeking behavior, potential secondary gain, and underlying psychologic and social stressors often confound a patient's self-report of pain. Health care providers are therefore keenly interested in a more objective method of assessing pain. Imaging methods that can quantitatively measure metabolic activity associated with particular states, such as

Table 3. Statistical Parametric Mapping Heat Pain

X	Y	Z	Cluster Size	Anatomy I	Anatomy II	t-Value
Activation						
60	-40	26	136	R Supramarginal gyrus	IPC (PF)	6.79
—	—	—	—	—	IPC (PFm)	—
4	31	0	96	R Anterior cingulate cortex	(ACC)	4.63
0	27	22	—	L Anterior cingulate cortex	(ACC)	5.46
-54	-68	4	87	L Middle temporal gyrus	—	6.35
-57	-47	30	79	L Supramarginal gyrus	IPC (PFm)	6.53
—	—	—	—	—	IPC (PFcm)	—
-32	-17	62	15	L Precentral gyrus	BA 6	7.82
27	16	-2	14	R Putamen	—	4.8
-42	-18	14	16	L Rolandic operculum	OP 1	4.7
—	—	—	—	—	OP 4	—
Deactivation						
51	-6	56	3	R Middle frontal gyrus	—	4.9

BA 6 = Brodmann area 6; IPC (PFcm)^{39,40} = inferior parietal cortex area PFcm; IPC (PFm)^{39,40} = inferior parietal cortex area PFm; L = left; OP 1 = parietal operculum area 1; OP 4 = parietal operculum area 4; R = right.

Table 4. Statistical Parametric Mapping Ischemic Pain

X	Y	Z	Cluster Size	Anatomy I	Anatomy II	t-Value
Activation						
-57	-29	18	95	L Superior temporal gyrus	OP 1	6.29
—	—	—	—	—	IPC (PFop)	—
-36	9	10	88	L Insula lobe	—	9.66
-45	32	9	18	L Inferior frontal gyrus	BA 45	4.99
16	64	0	18	R Superior orbital gyrus	—	6.75
5	-55	-67	14	R Middle temporal gyrus	hOC5 (V5)	5.11
9	-18	22	6	R Thalamus	—	4.65
Deactivation						
27	-30	66	15	R Precentral gyrus	BA 6	>4.3
34	-4	65	8	R Middle frontal gyrus	BA 4p	5.84
26	26	-68	6	R Superior occipital gyrus	SPL (7p)	>4.3
21	-87	-12	6	R Cerebellum	hOC3v	>4.3
24	-5	-14	4	R Amygdala	Amy (SF)	>4.3
-52	-27	64	2	L Postcentral gyrus	—	>4.3
10	14	32	2	R Anterior cingulate cortex	—	>4.3

Amy (SF) = amygdala superficial; BA 45 = Brodmann area 45; BA 6 = Brodmann area 6; BA 4p = Brodmann area 4p; hOC3y = human occipital lobe area 3y; hOC5 (V5) = human occipital cortex area 5; IPC (PFop) = inferior parietal lobe area PFop; L = left; OP 1 = parietal operculum area 1; R = right; SPL (7p) = superior parietal lobe area 7p.

CASL, probably offer the best hope for imaging biomarkers of acute and possibly also chronic painful conditions.

Comparison of ROI-based versus Global (SPM) Analysis

SPM analysis revealed a number of regional findings similar to the findings in the ROI-based analysis, but they appear more sporadic in terms of which pain condition elucidates them. As noted previously, SPM analysis showed “deactivation” in several regions (Brodmann area 6 and postcentral

gyrus) during the ischemic task that was consistent with the absolute rCBF reduction seen on ROI analysis. SPM also showed “activation” in the thalamus and insula in the ischemic condition, regions showing less rCBF reduction (than the mean) in the ROI analysis. Though clearly not identical in terms of statistical significance across all findings, our direct ROI analysis and the SPM analysis of normalized whole brain data show a great deal of overlap. We presume that differences are due to a combination of factors, including

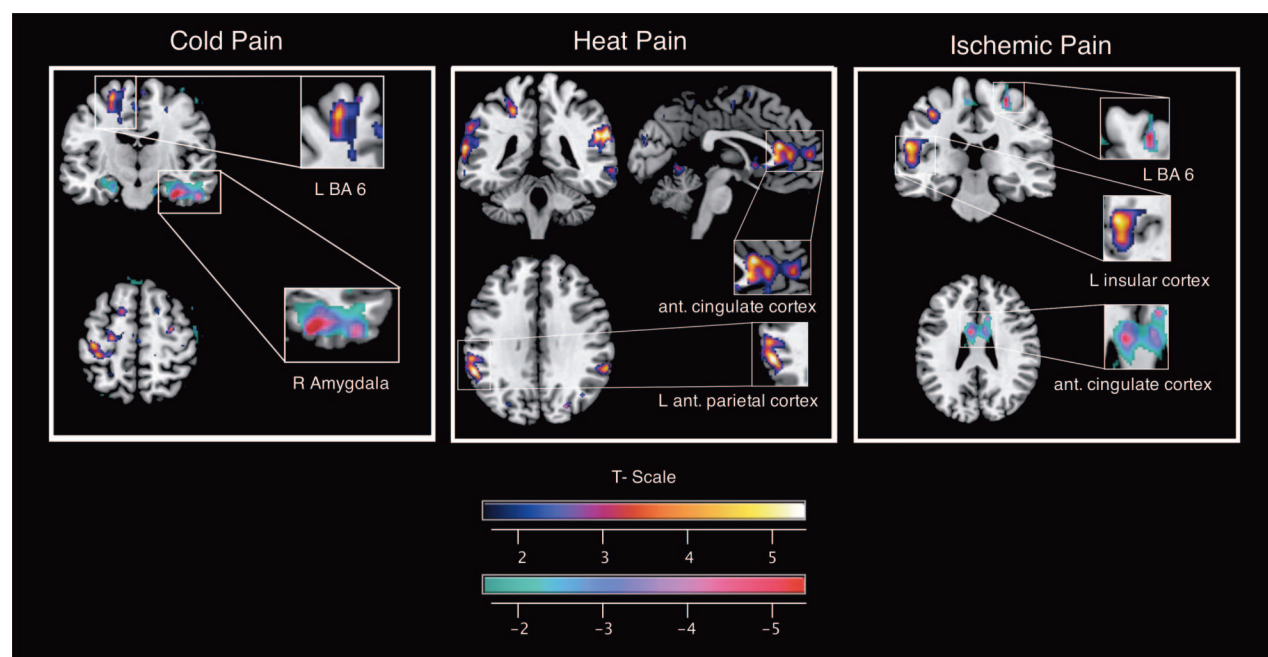


Fig. 4. Representative maps of increased and decreased rCBF for the cold pain, heat pain, and ischemic pain condition based on the SPM analysis. Relative signal change are coded using the National Institutes of Health (NIH)-fire color scheme (upper bar) for increase and the NIH-ice color scheme for rCBF reduction (lower bar). BA = Brodmann area; rCBF = regional cerebral blood flow; SPM = statistical parametric mapping.

SPM's adjustment for global changes and to the more conservative thresholds that need to be used when evaluating the entire brain, combined with the lower signal associated with the CASL (as opposed to BOLD fMRI) method. Surprisingly, SPM analysis of the whole brain did not reveal additional findings to the ROI analysis, attesting in part to the relative theoretical completeness of the ROIs examined, the relatively conservative thresholds applied to whole brain data, and perhaps the lower signal strength of ASL masking weaker activation effects.

The CASL imaging method used here appears to have great potential for the comparison of within-subject changes associated with state changes or interventions and between-subject baseline differences in rCBF. Future studies using this technique in targeted clinical pain populations are likely to reveal patterns of CBF that prove to have clinical utility on both the group and individual case level.

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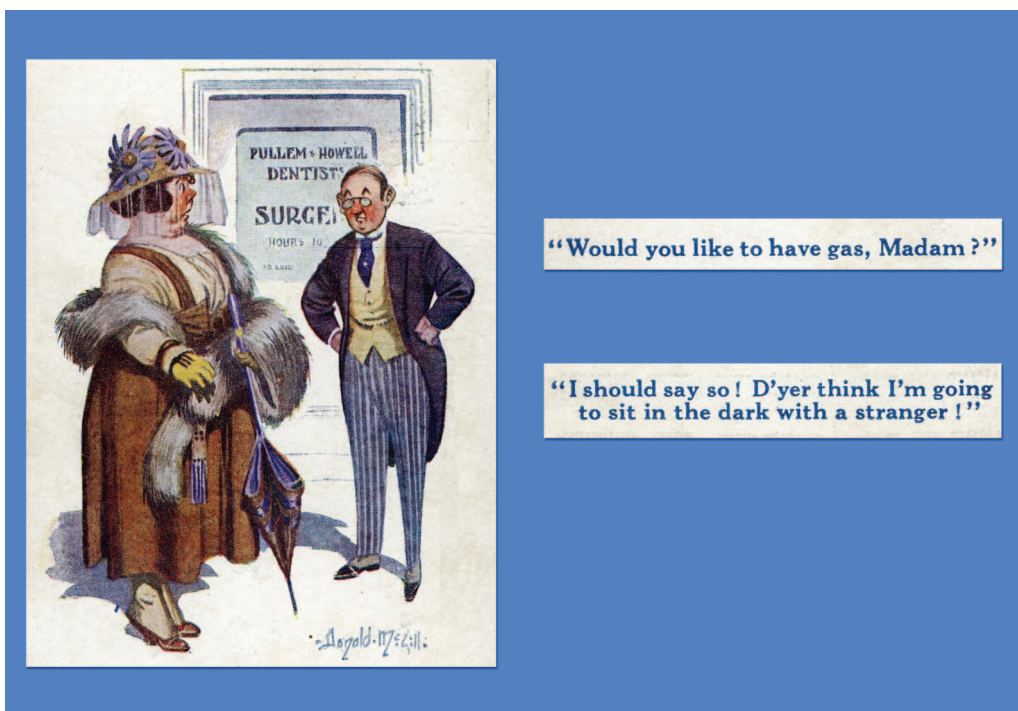
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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

A Dental Anesthetic for Tolerating a Stranger in the Dark?



“Would you like to have gas, Madam?”

“I should say so! D’yer think I’m going to sit in the dark with a stranger!”

About 9 months after the end of World War I, a visitor to the English seaside affixed a red one-penny King George V stamp to a mailing illustrated by “the king of the saucy postcard,” Donald McGill (1875–1962). After canceling the stamp on August 20, 1919, the Royal Mail managed to deliver the postcard without postmarking McGill’s artwork (*left*), which featured an overdressed woman outside the surgery of “Pullem & Howell, Dentists.” She is portrayed as insisting on receiving nitrous oxide in order to tolerate sitting “in the dark with a stranger” (*right*). McGill has apparently identified yet another use for laughing gas . . . (Copyright © the American Society of Anesthesiologists, Inc.)

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