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Pain Response as Measured with Arterial Spin Labeling

Nasim Maleki, PhD¹, Jennifer Brawn, BA¹, Gabi Barmettler, BA¹, David Borsook, MD, PhD^{1,2,3}, and Lino Becerra, PhD^{1,2,3}

¹Center for Pain and the Brain, Department of Anesthesia, Boston Children's Hospital and Harvard Medical School, Boston, MA

²P.A.I.N. Group, Department of Psychiatry, McLean Hospital and Harvard Medical School, Charlestown, MA

³Departments of Psychiatry and Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA

Abstract

The majority of functional magnetic resonance imaging studies of pain processing in the brain use the Blood Oxygenation Level Dependent (BOLD) imaging approach. The BOLD signal however is complex as it depends on simultaneous changes in blood flow, vascular volume and oxygen metabolism. Arterial Spin Labeling (ASL) perfusion imaging is another imaging approach in which the magnetically labeled arterial water is used as an endogenous tracer that allows for direct measurement of cerebral blood flow. In this study we assessed the pain response in the brain using a pulsed-continuous arterial spin labeling (pCASL) approach and a thermal stimulation paradigm. Using pCASL, response to noxious stimulation was detected in somatosensory cortex, anterior cingulate cortex, anterior insula, hippocampus, amygdala, thalamus and precuneus consistent with the pain response activation patterns detected using BOLD imaging approach. We suggest that pCASL is a reliable alternative for fMRI pain studies in conditions in which blood flow, volume or oxygen extraction are altered or compromised.

Keywords

Pain; Magnetic Resonance Imaging; fMRI; Arterial Spin Labeling; Arterial Spin Tagging; Perfusion; Hemodynamics; Blood Flow

INTRODUCTION

Functional Magnetic Resonance Imaging (fMRI) is a noninvasive technique that is widely used for assessing brain functions. The majority of fMRI-based studies use the Blood-Oxygenation Level Dependent (BOLD) technique that is sensitive to the relative level of oxy- and deoxy-hemoglobin concentrations in the blood as a result of brain activity induced changes in blood flow, blood volume and metabolic rate of oxygen extraction (1). This complex nature makes BOLD susceptible to producing signals that may not correspond to the underlying neurophysiology; for instance, blood flow and blood volume changes may be altered in disease or pharmacological conditions and influence the resulting BOLD signal.

In recent years arterial spin labeling (ASL) techniques have emerged as a technique for measuring cerebral blood flow noninvasively (2,3). ASL employs the spatial selectivity of

MRI to saturate or invert the spins of the hydrogen nuclei in blood before they enter the tissue of interest. If sufficient time is allowed for the labeled blood to flow into the tissue, a small intensity change in the MR image of the tissue will be observed. Because the water contained within blood readily diffuses across the blood-tissue barrier, the label acts as a diffusible tracer, a type of tracer with excellent properties for quantifying flow. A well-developed theory for quantifying flow with ASL has been established (3,4) and blood flow values in the brain have been validated with microspheres in animals (5) and with positron emission tomography in humans (6). To date, the application of ASL for functional imaging has been less widespread mainly due to technical difficulties associated with low signal-to-noise ratio (less than 1%) (4), limited whole brain coverage in most scanners, lower resolution, difficulty in implementing functional ASL on clinical scanners, and the numerous variants of the technique. This pattern however is changing (7).

While ASL can in theory be used in a variety of functional imaging studies, this paper only focuses on application of ASL to pain imaging. In recent years various studies have looked into using ASL in measuring blood flow changes in the brain in response to various forms of induced pain (see Table 1). Most of these studies have employed pulsed ASL techniques. In this paper we present results of pain related perfusion changes by combining a simple thermal stimulation paradigm and pulsed-continuous arterial spin labeling technique pCASL also known as pseudo-continuous arterial spin labeling (8,9). pCASL is a practical and efficient solution for continuous labeling on systems with only pulsed RF capability i.e. clinical MR scanners (10,11) and unlike continuous techniques does not require special hardware. In this approach, continuous labeling of the arterial blood is achieved through employing rapidly repeated gradient and radio frequency pulses that mimic continuous labeling by flow-driven adiabatic inversion. This strategy has a higher signal to noise ratio and labeling efficiency compared to pulsed techniques such as (FAIR, PICORES, Q2TIPS) (12–15). In this paper we will present a simple paradigm and will show how cerebral blood flow changes could be measured using the pCASL approach.

EXPERIMENTAL

Subjects

The study was approved by the Institutional Review Board, and it met the criteria of the Helsinki accord for experimentation of pain in human subjects (<http://history.nih.gov/research/downloads/helsinki.pdf>) and approved informed consent forms were obtained from all subjects. Twenty healthy subjects were screened and underwent quantitative heat sensory testing (QHST) in order to determine their pain threshold temperature for the pain sensation at a level of 7 out of 10 on a scale from 0 (no pain) to 10 (maximum imaginable pain). QHST was performed using a 30×30 mm² contact thermode (TSA-II, Medoc Advanced Medical Systems, Ramat Yishai, Israel) that was placed on the dorsum of left hand. The temperature was ramped at a rate of 1°C/sec from a baseline of 32°C, subjects were instructed to stop the procedure at the onset of pain. This was repeated 3 times and the temperatures were averaged. Subjects who had very high (>48°C) or very low pain thresholds (<42°C) were not included in the study. Twelve subjects (6 female) were enrolled in the imaging study and were matched for age and gender.

Imaging

All imaging data were collected on a 3 Tesla Siemens Trio scanner with an 8-channel phased array head coil (Erlangen, Germany). For structural data, high resolution, T₁-weighted datasets were collected from each subject using a 3D MPRAGE pulse sequence (TR/TE/TI=2100/2.74/1100ms, FA=12, 128 sagittal slices, voxel size = 1.33 × 1.0 × 1.0 mm³). Functional images were acquired using a pulsed-continuous ASL (pCASL) perfusion

imaging sequence (8,9) using body coil transmission and head coil reception. For pCASL, an RF pulse train of 1500ms duration was applied 9cm beneath the center of the acquired slices, with a mean gradient of 0.6mT/m, RF duration of 500 μ s, a gap of 360 μ s, and flip angle of 25°. Other parameters were field-of-view=240×240 mm², matrix=64×64, 16 slices acquired in ascending order, slice thickness=5 mm, gap between slices=1.5mm, post labeling delay=1200ms, TR=4000ms, TE=18ms, gradient-echo echo-planar readout with 45ms readout time per slice.

Subjects underwent a functional scan with stimulation at 46°C using the same thermode used for QHST that was applied to the same area of the left hand. During functional imaging, 8 blocks of stimulation (duration: 15s) were delivered from a baseline temperature of 32°C at 30s intervals, which corresponded to 8×(15 s stimulation + 30 s baseline). The rate of temperature change was 4°C/sec; therefore, a total ramp time of 56 sec was accounted for which corresponds to $((46^{\circ}\text{C} - 32^{\circ}\text{C}) \times (8 \text{ stimulation} \times 2 \text{ ramps each}))/4^{\circ}\text{C}$. The number of controls/labels was 51 pairs and 2 dummy scans were also acquired for signal stabilization that were omitted in the preprocessing step. Therefore, an extra time of $2 \times \text{TR} = 8 \text{ sec}$ was added to the duration of the first 32°C interval at the beginning of the stimulation paradigm to account for that. A baseline (no stimulation) perfusion scan was also performed with the number of controls/labels = 30 pairs.

Analysis

FSL (<http://fsl.fmrib.ox.ac.uk/fsl/>) analysis tools from Oxford center for functional magnetic resonance imaging of the brain (fMRIB) were used for voxel-based statistical analysis of the functional data. For each subject, all of the functional data were motion corrected using MCFLIRT tool, skull stripped using BET tool, smoothed with a Gaussian 5 mm full-width at half-maximum filter to reduce spatial variability, and filtered by applying a 60 s high-pass temporal filter. First-level fMRI analysis of single subject data was performed using FMRI Expert Analysis Tool (FEAT) Version 5.98. The explanatory variables (EV) for thermal stimuli were setup according to stimulation timings, and then convolved using a standard hemodynamic response function (HRF) convolution for each subject. Odd time points were subtracted from even time points in order to convert control-label alternating time points into a perfusion-only signal. For comparison a BOLD signal was generated from label and control images, so that each BOLD signal time point was an average of the consecutive label and control images. Group activation maps were generated by fMRIB's Local Analysis of Mixed Effects (FLAME1) tool, which uses Bayesian modeling and estimation for detecting activation. Group activation statistical maps were thresholded at a posterior probability of ($p > 0.05$) using a Gaussian Mixture Model (GMM) technique (16) and then clusters were thresholded with a minimum cluster criterion of 3×3×3 voxels in MNI space.

Spatial Normalization

All of the anatomical and functional data were spatially normalized to the MNI152 brain for group analysis provided with the FSL package. Spatial Normalization was performed using FLIRT following a two-stage process: 1- low-resolution functional image linearly registered to the high-resolution structural image. 2- linear (affine) registration of the high-resolution structural image to the standard MNI 152 average brain.

Blood Flow Quantification

The signal difference between label and control images depends on the blood flow as well as other parameters such as blood-tissue partition coefficient, T1 of blood and tissue, the efficiency of spin labeling and the transit time to the imaging region. Blood flow was quantified using the following equation that assumes a well-mixed single compartment model (17):

$$f = \frac{\lambda R_1 \Delta M}{2\alpha M_0 (e^{-wR_1} - e^{-(\tau+w)R_1})}$$

where ΔM is the time-averaged perfusion-weighted signal, M_0 is the equilibrium brain tissue magnetization; f is blood flow; λ is the blood/tissue water partition coefficient; R_{1a} : longitudinal relaxation rate of blood; α : labeling efficiency; τ : labeling duration and w post-labeling delay. We assumed $\alpha=0.85$, $\lambda=0.9$ g/ml (18), $R_{1a}=0.67$ s⁻¹ (19), $w=1200$ ms and $\tau=1500$ ms for all subjects.

In order to determine the magnitude of the blood flow changes in response to noxious thermal stimulation, the time-averaged perfusion signal for the stimulation 'on' periods as well as the baseline periods was calculated. The blood flow was estimated for each condition and the difference between the two states was determined. The difference maps were transformed to the standard space and averaged across the subjects.

RESULTS

Pain Measures

All of the participants (N=12, 6 male, age: 29.9 ± 2.5 (mean $\pm$ std) years) successfully completed the study. The average 7 out of 10 pain threshold temperature was $45.8 \pm 1.6^\circ\text{C}$ (mean $\pm$ std). The pain threshold temperature was slightly higher in male ($46.9 \pm 1.6^\circ\text{C}$ (mean $\pm$ std)) vs. female ($45.1 \pm 1.2^\circ\text{C}$ (mean $\pm$ std)) participants but the difference was not significant ($p < 0.08$). All of the subjects were feeling pain at 46°C and on a scale from zero to ten (0: no pain at all, 10: maximum pain imaginable) the average pain rating was 6.0 ± 2.2 (mean $\pm$ std) (male: 5.9 ± 1.6 (mean $\pm$ std), female: 6.0 ± 1.9 (mean $\pm$ std)).

Imaging Measures

Examples of baseline cerebral blood flow maps are shown in Figure 2 for all of the subjects who participated in the study. The images represent the quantified blood flow values in ml/100gr/min. The contrast between highly perfused cortex (and cerebellum) and white matter is easily seen in these images. Group average ASL activation in response to thermal stimulation on the left hand at 46°C are presented in coronal and axial view in Figure 3. There was a significant response ($p < 0.01$) to noxious stimulation at 46°C in structures such as insula, thalamus, hippocampus, amygdala, anterior cingulate, primary and secondary somatosensory cortex, precentral gyrus and in precuneus, Table 2.

The blood flow changes between pain and baseline conditions were measured in each subject separately and the average across all of the subjects was calculated in standard space. The results are shown in Figure 4. An increase in CBF was observed in anterior cingulate, anterior insula, frontal pole, posterior cingulate, hippocampus and precuneus. For comparison, BOLD activation maps are presented in Figure 5. Significant BOLD response was detected in supramarginal cortex, middle frontal cortex, posterior cingulate, anterior cingulate, thalamus and basal ganglia.

DISCUSSION

Variations in neural activity require delivery of oxygen and nutrients to neuronal tissue that is controlled by perfusion at a microvascular level i.e. capillaries. As such, functional activation of the brain regions can be measured indirectly by measuring changes in the regional cerebral blood flow using PET or measuring the blood oxygen level dependent

changes in fMRI. In this study a pCASL perfusion imaging technique was used to measure the focal brain responses to painful stimulation.

Using pCASL technique, we found increases in blood flow to noxious stimuli in areas that are almost consistently observed in BOLD fMRI and PET studies (20,21). We found activation of the thalamus, primary somatosensory cortex (S1) and insula, regions thought to be related to the sensory-discriminative aspects of pain processing. More notably we found profound increases in the blood flow in hippocampus and precuneus/posterior cingulate that are hubs of the default mode network. Owen et al., (22) had noted increase in blood flow in precuneus in response to noxious stimulation on the hand. A recent study (23) has suggested a gender-specific role for precuneus in migraine, a neurological disorder characterized by painful headaches, where female migraineurs have a thicker cortex in the precuneal region associated with higher perfusion (24). Posterior cingulate cortex is also associated with a variety of functions including memory retrieval (25), emotion (2), pain (3), and resting state. A recent study has shown that default mode network activity in response to pain is biphasic, deactivated during anticipation for pain and activated during pain perception (26). Our findings of significantly increased blood flow in the precuneus/posterior cingulate regions are consistent with these reports and provide the neurophysiologic proof of the mechanisms of this increase in activation. Finally, there are various reports suggesting a role for hippocampus in pain processing (27–33). More recent imaging studies of pain in humans report hippocampal activation in a variety of conditions (e.g., (34,35). The increase in blood flow in the amygdala in response to pain induced via balloon dilatation of a dorsal foot vein of healthy volunteers has been reported (36). This region is suggested to be involved in the affective dimension of pain. Similarly the same study reports correlation between increase of blood flow and subjective rating of pain (36).

The perfusion maps shown in Figure 2 show slightly higher baseline perfusion levels for some of the subjects compared to the others. Aside from normal physiologic variability among subjects, this could stem from various sources such as differences in the transit delay between subjects, or the differences in blood relaxation rate. However, for all of the subjects the measures were all well within the range of normal perfusion levels. The differences in estimates of baseline perfusion levels among subjects did not affect our analysis results. The correlation of signal changes in each voxel relative to the stimulation paradigm was assessed for each subject; therefore, the variability of the baseline values didn't affect the final results. Since the “change” in CBF in response to noxious stimulation was measured in the quantitative comparison, again the difference in baseline levels did not impact the analysis.

Since the primary aim of this study was to test the feasibility of performing pCASL in studying the pain response, we did not acquire T1 maps of the brain tissue or the transit delay to the tissue. Therefore for quantification of the CBF we made some assumptions: it was assumed the labeled blood arterial spins remain primarily in the vasculature rather than exchanging completely with tissue water and we assumed that arterial transit time (δ_a), post labeling delay (w), and tissue transit time (δ) fulfill the condition $\delta_a < w < \delta$. Dai et al. (17) had reported measurement of the cerebral arterial transit delay using CASL approach in healthy subjects in different regions of the brain. They report transit delay values ranging from 1.24 seconds in the inferior regions to 1.5 second in the superior regions of the brain. With regards to the arterial transit delay, Lu et al. have reported arterial transit delay of ~0.9 seconds (37). Therefore with the post labeling delay of 1.2 seconds the $\delta_a < w < \delta$ condition is satisfied. It should also be noted that the post-labeling delay depends on the slice position with regards to image acquisition order (ascending in our case) and the image acquisition time. This difference in post labeling delay was taken into account for blood flow quantification in each slice by splitting the 4D image volumes in axial (Z) direction and

quantifying the blood flow separately and then merging the volumes back together for the final analysis.

As presented in Table 1, there have been various studies on applying ASL techniques in studying pain responses in the brain. Most of the studies have used pulsed ASL approaches while mostly using noxious stimulation for evoking pain response. One recent study has used pCASL approach to measure CBF changes associated with ongoing pain post tooth extraction surgery. This study identified 5–10% increase in regional cerebral blood flow representing post-surgical ongoing pain in S1 and S2, insula and cingulate cortices, thalamus, amygdala, hippocampus, midbrain and brainstem (20). Froellich et al. (38) report the results of using a CASL approach for studying the effect of heat noxious stimulation on CBF changes in the brain. They report an increase in average CBF in insula of ~ 2 mL/100gr/min. Considering that the maps that we present in Figure 4 are voxel based and not ROI based, for comparison, we measured the average CBF change in an insula ROI derived using the Anatomical Automatic Labeling (AAL) atlas (39) on a standard MNI brain. We found an average CBF change of 2.76 ± 3.31 (mean $\pm$ std) mL/100gr/min, which is consistent with their findings. Increase in CBF in S1, insula and ACC in response to noxious stimulation in response to painful heat is almost reported in all of the studies listed in Table 1. We found common areas of increased blood flow similar to the studies described above as well as in additional regions including thalamus, secondary somatosensory cortices, pre-central gyrus, and occipital cortex as well as in the hippocampus, amygdala and temporal pole.

Finally, in this study activation patterns in response to noxious stimulation were presented using three approaches i.e. the statistical ASL response approach (Figure 3), the Δ CBF approach (Figure 4) and the statistical BOLD response approach (Figure 5). Differences between the ASL and BOLD response in terms of the spatial specificity are reported by various studies (40,41). Similarly it could easily be appreciated from our result figures that the activated areas in the statistical ASL approach appear smaller than that of BOLD or Δ CBF approach. This could stem from the intrinsic lower level of ASL signal change that affects the voxel-based statistics significantly or limited venous signal contribution to the ASL signal as compared to BOLD that makes the ASL response more focal and less spread out (40). On the other hand, however, Δ CBF activation map is comparable with BOLD response as the Δ CBF approach benefits from averaging the signal that enhances the ASL signal. It is needless to say that the Δ CBF approach has the unique benefit of producing quantitative results that the statistical approaches lack. While the activation maps acquired by all three approaches are essentially comparable, the choice of the analysis approach will depend on the objective of the analysis. For instance while assessment of the correlation of the response pattern to the stimulation paradigm using the statistical ASL approach may reveal abnormalities or disruptions in disease states in the brain with high spatial specificity, the Δ CBF may be a better choice from drug or longitudinal studies in order to acquire measures that are comparable at different time points.

In conclusion, our results indicate that pCASL MRI may be used to study brain processes of pain using a simple heat stimulation paradigm. Brain response to painful stimuli result in brain activation patterns similar to those obtained with BOLD. ASL might provide a more suitable imaging approach to measure brain activity in conditions in which blood flow, volume or oxygen extraction are altered (such as elderly people, drugs, diseases). It could also provide quantitative measures for changes that could be compared across different states or even longitudinally to assess the effect of a therapy, intervention or the course of a recovery.

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Abbreviations

ASL	Arterial Spin Labeling
ACC	Anterior Cingulate Cortex
BOLD	Blood Oxygenation Level Dependent
FEAT	fMRI expert analysis tool
FLAME	fMRIB's Local Analysis of Mixed Effects
fMRIB	Oxford center for functional magnetic resonance imaging of the brain
fMRI	Functional Magnetic Resonance Imaging
GMM	Gaussian Mixture Model
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo
NAc	Nucleus Accumbens
pCASL	Pulsed-Continuous Arterial Spin Labeling
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
QHST	Quantitative Heat Sensory Testing
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex
THR	Pain Threshold
WM	White Matter

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Figure 1. ASL Scheme

Schematic presentation of the principal concept of perfusion imaging where Inverted/ Saturated spins of inflowing arterial blood act as intrinsic contrast agent and cause reduction in net magnetization in the tissue downstream. The amount of reduction depends on the perfusion. The perfusion image results from subtracting two images, the label image and the control image. (The labeling depicted in this figure is how the labeling is performed in Continuous Arterial Spin Labeling (CASL) and pulsed CASL (pCASL)).

Figure 2. Baseline Cerebral Blood Flow Maps
CBF maps of all of the participants in the study.

Figure 3. ASL Activation

Group average ASL activation in response to thermal stimulation on the left hand at 46°C are presented in coronal and axial view. There was a significant response ($P < 0.01$) to noxious stimulation at 46°C in structures such as insula (Ins), thalamus (Thal), hippocampus (Hipp), anterior cingulate cortex (ACC), post central gyrus (PoC), LO (lateral occipital), supramarginal cortex (SM), and in precuneus (Precun).

Figure 4. CBF Changes in Response to Noxious Stimulation

ΔCBF maps show regions with increase in CBF at 46°C, averaged across all of the subjects. Significant blood flow changes were observed in insula (Ins), anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), precentral gyrus (PreC), frontal pole (FP), hippocampus (Hipp), operculum cortex (Operc), precuneus(Precun).

Figure 5. BOLD Activation

Group average BOLD activation in response to thermal stimulation on the left hand at 46°C is presented for comparison to ASL findings. There was a significant response ($P < 0.01$) to noxious stimulation at 46°C in structures such as insula (Ins), thalamus (Thal), pallidum (Pall) anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), precentral gyrus (PreC), frontal pole (FP), and supramarginal cortex (SM).

Table 1

Summary of Prior Reports Using ASL

Author	Method	Subjects	Stimulus	Location of Stimulation	Results
Froellich et al., 2012	CASL 3T	Healthy N=10 5 Males 5 Females	Heat Cold Ischemic Pain	Hand	Increases in: Cold: <ul style="list-style-type: none"> Supplemental motor cortex Amygdala S1 Heat: <ul style="list-style-type: none"> ACC Insula Inferior Parietal
Howard et al., 2012	pCASL 3T	Post-Surgical Pain Patients N=16 All male	None	NA	Increases in: S1, S2, Insula, Cingulate, Thalamus, Amygdala, Hippocampus, Midbrain, Brainstem
Owen et al., 2012	PASL 3T	Healthy N= 19 All Male	Hypertonic saline infusion	Forearm (Brachioradialis)	Increases in: <ul style="list-style-type: none"> Insula IFG MFG Rolandic operculum
Wasan et al., 2011	PASL 3T	Chronic pain patients N=16 Controls N=16	Movement Heat	Hip / Leg Affected leg	Increases in: <u>Patients</u> Movements: <ul style="list-style-type: none"> S1 Prefrontal cortex Insular cortex Superior parietal Heat: <ul style="list-style-type: none"> No changes <u>Controls</u> Movements: <ul style="list-style-type: none"> No changes

Author	Method	Subjects	Stimulus	Location of Stimulation	Results
					Heat: <ul style="list-style-type: none"> • Superior temporal • Posterior cingulate
Zeidan et al., 2011	PASL 1.5 T	N=15 6 Males 9 Females	Heat	Leg (Calf)	Increases in: <ul style="list-style-type: none"> • ACC • Insula • S1 and S2 • Frontal operculum
Owen et al., 2008	PASL 3T	Healthy N = 14 13 Males 1 Female	Heat	Hand	Increases in: <ul style="list-style-type: none"> • Insula • S1 and S2 • Cingulate cortex • Thalamus • Precuneus • Superior parietal • Superior occipital

IFG: Inferior Frontal Gyrus, MFG: Middle Frontal Gyrus, ACC: Anterior Cingulate Cortex, S1: Primary Somatosensory Cortex, S2: Secondary Somatosensory Cortex

Table 2

Group Average ASL Activation in Response to Noxious Stimulation (46°C)

Brain Region	Lat.	z-stat	X(mm)	Y(mm)	Z(mm)	Vol(cm ³)
Postcentral	R	2.1596	48	-12	32	0.24
	R	1.9789	50	-12	38	0.96
SupraMarginal	R	2.3797	46	-32	44	0.296
Inferior Parietal	L	3.1083	-30	-42	44	1.576
	L	2.1199	-50	-28	42	0.256
Precuneus	L	2.7774	-4	-48	46	7.256
Rolandic Operculum	R	2.7404	46	-10	14	0.816
Lateral Occipital	R	2.4227	34	-72	20	0.328
	L	2.235	-26	-72	26	0.24
	R	2.3114	42	-82	8	0.408
	R	1.9809	28	-86	10	0.328
	L	2.3221	-30	-86	24	0.48
Temporal Pole	R	2.3192	32	8	-38	0.216
Middle Temporal	L	2.0504	-64	-10	-2	0.224
Superior Temporal	L	2.3541	-60	-22	12	0.68
Cingulate		2.1249	0	2	42	0.226
Insula	R	2.7404	40	-10	12	0.816
	L	2.3194	-42	-12	4	0.328
Hippocampus	R	2.5337	26	-10	-18	0.224
Amygdala	L	2.3873	-18	-10	-16	0.213
Thalamus	R	2.4621	18	-22	2	0.592
	L	2.087	-20	-34	4	0.24