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# Test-retest reliability of evoked heat stimulation bold functional magnetic resonance imaging

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**TEST-RETEST RELIABILITY OF EVOKED HEAT STIMULATION BOLD  
FUNCTIONAL MAGNETIC RESONANCE IMAGING**

by

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B.A., Augustana College, 2012

Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

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# **TEST-RETEST RELIABILITY OF EVOKED HEAT STIMULATION BOLD**

## **FUNCTIONAL MAGNETIC RESONANCE IMAGING**

**JORDAN DANIEL LEMME**

### **ABSTRACT**

To date, the blood oxygenated-level dependent (BOLD) functional magnetic resonance imaging (fMRI) technique has enabled an objective and deeper understanding of pain processing mechanisms embedded within the human central nervous system (CNS). In order to further comprehend the benefits and limitations of BOLD fMRI in the context of pain as well as the corresponding subjective pain ratings, we evaluated the univariate response, test-retest reliability and confidence intervals (CIs) at the 95% level of both data types collected during evoked stimulation of 40°C (non-noxious), 44°C (mildly noxious) and a subject-specific temperature eliciting a 7/10 pain rating. The test-retest reliability between two scanning sessions was determined by calculating group-level interclass correlation coefficients and at the single-subject level.

Across the three stimuli, we initially observed a graded response of increasing magnitude for both visual analogue scale (VAS) pain ratings and fMRI data. Test-retest reliability was observed to be highest for VAS pain ratings obtained during the 7/10 pain stimulation (intraclass correlation coefficient (ICC) = 0.938), while ICC values of pain fMRI data for a distribution of CNS structures ranged

from 0.5 to 0.859 ( $p < 0.05$ ). Importantly, the upper and lower CI bounds reported herein could be utilized in subsequent trials involving healthy volunteers to hypothesize the magnitude of effect required to overcome inherent variability of either VAS pain ratings or BOLD responses evoked during innocuous or noxious thermal stimulation.

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## LIST OF ABBREVIATIONS

BOLD	blood oxygenated-level dependent
BU	Boston University
CI	confidence intervals
CNS	central nervous system
EV	explanatory variable
fMRI	functional magnetic resonance imaging
FA	Flip Angle
fBIRN	functional Biomedical Informatics Research Network
FOV	Field of View
GE-EPI	gradient echo-echo planar pulse sequence
GLM	general linear model
ICC	intraclass correlation coefficient
ISO	International Standards Organization
MPRAGE	Magnetization-Prepared Rapid Acquisition Gradient-Echo
MRI	Magnetic Resonance Imaging
QU	quality assurance
QST	quantitative sensory testing
RCMP	Royal Canadian Mounted
ROI	regions of interest
SNR	signal to noise ratio
SOP	standard operating procedures

TE ..... Time of Echo  
IT ..... Inversion Time  
TR..... Time of Repetition  
TSA..... Thermal Sensory Analyzer  
VAS .....visual analogue scale

## INTRODUCTION

With blood oxygenated-level dependent (BOLD) functional magnetic resonance imaging (fMRI) methodology, the behavior of pain pathways structures within the central nervous system (CNS) has been characterized in states of acute or chronic pain [7; 8; 11], during therapeutic intervention [4; 18; 21] and during the placebo response [1; 6]. From work stemming from the pain fMRI field, it is better known which CNS structures can potentially be targeted, directly or indirectly, in order to obtain a possible analgesic effect (e.g., insula in fibromyalgia patients) [5]. However, despite the large body of pain fMRI work, there remain fundamental unknowns regarding the utility of this methodology, as a means to characterize pathology in distinct pain patient populations or to evaluate novel therapies, be they pharmacological or behavioral in nature. One area believed to require further attention is a more indebt assessment of test-retest reliability of pain fMRI experimental paradigms [9; 13]. These studies mainly have looked at repeated exposure of subjects to the same stimulus. With test-retest reliability of pain fMRI in hand, its utility particularly during longitudinal, proof-of-concept clinical trials can be further realized.

In this study, to better reflect a randomized clinical trial with a cross-over design of drug and placebo, subjects were enrolled in a drug (buprenorphine) placebo study, however, they only received saline injections. We aimed to quantify the reproducibility of the pain fMRI method itself so that in future work, variations in

the BOLD fMRI signal can be more confidently attributed to inherent fluctuations in pain levels perceived in patients, treatment effect or a combination of the two.

Across pain fMRI studies, the stimulation paradigm has indeed varied from study to study. These variations derive from study site preferences as well as which pain stimuli may be most suitable to implement in order to probe the etiology and pathology of a particular patient population. However, thermal heat pain is one type of evoked stimulation widely used in not only fMRI studies, but also behavioral work aimed solely at quantitative sensory testing (QST) in healthy subjects or pain patients. To date, it is well known that thermal heat pain induces robust BOLD activation and deactivation in multiple ascending and descending pain pathway structures, which process sensory and affective components of pain [19]. Multiple studies have dissected the properties of the BOLD signal elicited by noxious thermal stimuli and in turn, have characterized what features (e.g., biphasic response) of the BOLD signal are specific to evoked pain [10; 12; 19]. Moreover, cross-sectional investigations utilizing thermal heat pain have clearly demonstrated that a substantial dynamic range exists in the BOLD signal such that a treatment effect, acute or chronic, can be measured.

Given the *a priori* knowledge of thermal pain responses as measured by BOLD fMRI methodology, this healthy subject study focused on a quantitative assessment of test-retest reliability of pain fMRI during the presence of heat

stimuli. Based on the variability of what temperatures are considered painful from one individual to another, a subject-specific heat stimuli yielding a 7 out of 10 rating on the visual analogue scale (VAS) was utilized. The objective of our study was to further evaluate the test-retest reliability of pain fMRI by calculating the intraclass correlation coefficient (ICC) across CNS structures previously implicated in pain processing. The ICCs across brain regions were compared for 40°C, 44°C and subject-specific temperatures corresponding to 7/10 pain rating, where the latter was considered the 'pain condition'. Furthermore, the current study was carried out with the specific needs and requirements of a pharmacological fMRI study in mind [14; 15]. Therefore, steps enabling better standardization of study procedures were executed as well as onsite 'drug' administration of a placebo pill at each scan session.

## METHODS

### Study Participants

This investigation was approved by the McLean Hospital Institutional Review Board

Twelve healthy, right-handed male participants were included in this study (mean age  $\pm$  standard deviation: 31  $\pm$  8.8 years old). Of these 12 study participants, 10 individuals possessed complete imaging + behavioral datasets that are reported on herein. Each of the 10 subjects made three study site visits (prescreen and 2 MRI scanning sessions termed Scans 1 and 2). The prescreen visit consisted of the following procedures: 1) Introduction to 40°C and 44°C stimuli (dorsum or left foot) in order to become familiar with the Medoc Thermal Sensory Analyzer (TSA) probe and stimuli itself, 2) QST to determine the subject-specific temperature corresponding to a 7 out of 10 pain rating on the VAS (maximum temperature = 50°C), 3) Training on pain reporting equipment utilized during fMRI, 4) Review of complete medical history and 5) Physician assessment, which included physiological measurements.

Enrolled participants had no presence of physical or mental illness, no presence chronic pain disorders, consumed no medications and reported no history of alcoholism or drug abuse. Subsequent to prescreening procedures, each

individual underwent 2 scanning sessions that were ~2-3 weeks apart. All volunteers gave informed consent prior study participation. All volunteers were told that they would receive either saline (placebo) or an analgesic infusion in a cross-over design. After they finished participating in the study they were told that only saline was used.

### **Pre-Scanning Procedures**

For each subject and each scanning session, a detailed checklist was kept to ensure all predefined standard operating procedures (SOP) were adhered to. Within this checklist, deviations from the SOP were logged (e.g., ancillary equipment malfunction) as well as timestamps of distinct components of the study (e.g., baseline vitals or start of anatomical MRI). Drug test were first completed before each scan session to verify the subjects' eligibility. Following drug testing, subjects received 1) A brief re-training of VAS pain rating equipment and review of the study procedures, 2) Physician assessment, 3) Placement of an intravenous line (subjects' left arm) for subsequent within MRI scanner blood draw (not actually performed) and 4) Oral drug administration (sublingual vitamin B-12 pill) prior to subject placement within the scanner. Once subjects were positioned within the MRI monitoring of physiology (heart rate, end-tidal CO<sub>2</sub>, PO<sub>2</sub>, respiratory rate) was initiated.

## **Heat Stimulation**

During the fMRI scan, a mixed heat stimulation paradigm was used to assess the reproducibility of 40°C (non-noxious), 44°C (mildly noxious) and the subject-specific, 7/10 noxious heat pain stimulation. A 32 cm<sup>2</sup> TSA thermode was attached to the subjects left foot via Velcro strap with a baseline temperature of 32°C. The stimulation paradigm consisted of 25 second off and 15 second on cycle where each temperature (i.e., 40°C, 44°C and 7/10) was randomly presented five times. During heat stimulation and fMRI data collection, VAS pain ratings were continuously and simultaneously collected from the subjects.

## **MRI Data Acquisition**

MRI data were collected on a 3 Tesla Siemens Trio scanner with an 8-channel phased array head coil (Erlangen, Germany). fMRI data were collected using a gradient echo-echo planar pulse sequence (GE-EPI) at a 3.5 x 3.5 x 3.5 mm<sup>3</sup> resolution. GE-EPI Parameters: Time of Repetition (TR) = 2500 msec, Time of Echo (TE) = 30 msec, Field of View (FOV) = 224x224, Flip Angle (FA) = 90°, # of Slices = 41 axial slices, # of Volumes = 283. Magnetization-Prepared Rapid Acquisition Gradient-Echo (MPRAGE) anatomical images were collected. MPRAGE Parameters: TR = 2100 msec, TE = 2.74 msec, Inversion Time (TI) = 1100 msec, FA = 12°, 128 sagittal slices.

### **Quality Assurance (QA) of MRI Data**

QA of all fMRI data was performed using a MatLab (MathWorks, Sherbon, MA, USA)-based functional Biomedical Informatics Research Network (fBIRN) analysis algorithm (fBIRN-qa-calc\_birn.m). Each fMRI dataset was also motion corrected using FSL's McFlirt algorithm (FMRIB Software Library (FSL 5) ([www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl))), and evaluated to determine if the maximum deviation due to motion was never greater than 2.5 millimeters (mm), the in-plane resolution utilized during fMRI data acquisition.

### **fMRI Data Processing**

Subsequent single-subject fMRI data analyses were performed using FSL 5 [25]. Preprocessing steps for fMRI data have been described elsewhere [17; 19]. Moreover, a dual explanatory variable (EV) general linear model (GLM) analysis of single-subject fMRI data was utilized to quantify early (stimulus-locked) and late phase BOLD responses identified and characterized in earlier evoked pain fMRI work. Within group average analyses were performed using a mixed-effects analyses [22]. Each group average statistical map was set to a threshold of  $z > 2.3$  and cluster-size corrected [16].

### **Intraclass Correlation Coefficients and Confidence Intervals**

To evaluate the test-retest reliability of VAS pain rating and BOLD fMRI data between Scans 1 and 2 and data stemming from 40°C, 44°C and 7/10 heat stimuli, the ICC values were calculated using SPSS v21.0 (SPSS Inc., Chicago, IL, USA). For BOLD fMRI data, multiple regions of interest (ROI, **Table 1**) were selected using data provided in earlier pain fMRI work [3] as a guide and anatomically defined using the WFU PickAtlas (WFU Pickatlas, v2.4). The PickAtlas ROI for mid-cingulum was modified based on earlier findings within this structure [20]. The sensory thalamus ROI was defined using FSL's DTI-based segmentation of the thalamus. The ROI corresponding to the foot representation within the primary somatosensory cortex has been defined previously. From each atlas defined ROI, the mean BOLD response defined in terms of parameter estimates were extracted from each subject and each scanning session. ICCs of absolute agreement were then calculated using a two-way mixed model that provided a measure of consistency through a ratio of between subject variance to total variance [2]. This statistical practice has been also been used in recent work aiming to test the reliability of fMRI data [9]. In addition, CIs at a 95% confidence level were calculated for all VAS pain ratings and all ROI evaluated.

**Table 1: Regions of interest for which evoked BOLD responses and test-retest reliability were quantified during 40°C, 44°C and 7/10 thermal pain stimulation paradigms.** Quantification was performed in left and right components of each ROI unless specified otherwise. \*Quantification of metrics from the primary somatosensory cortex was restricted to the right hemisphere (contralateral to stimulation site) and foot representation.

Regions of Interest	
Amygdala	Inferior Frontal Triangular
Anterior Cingulate	Middle Frontal
Anterior Insula	Middle Frontal Orbitalis
Caudate	Middle-Anterior Cingulate
Cerebellum Crust I	Middle-Posterior Cingulate
Cerebellum Crust II	Nucleus Accumbens
Cerebellum III	Posterior Cingulate
Cerebellum IV & V	Posterior Insula
Cerebellum IX	Primary Somatosensory Cortex (right)*
Cerebellum VI	Putamen
Cerebellum VIIb	Sensory Thalamus (bilateral)
Cerebellum VIII	Superior Frontal
Cerebellum X	Superior Frontal Orbitalis (left)
Hypothalamus	Superior Medial Frontal
Inferior Frontal Opercularis	Supplemental Motor Area
Inferior Frontal Orbitalis	Thalamus

## RESULTS

### Subjective Pain Ratings

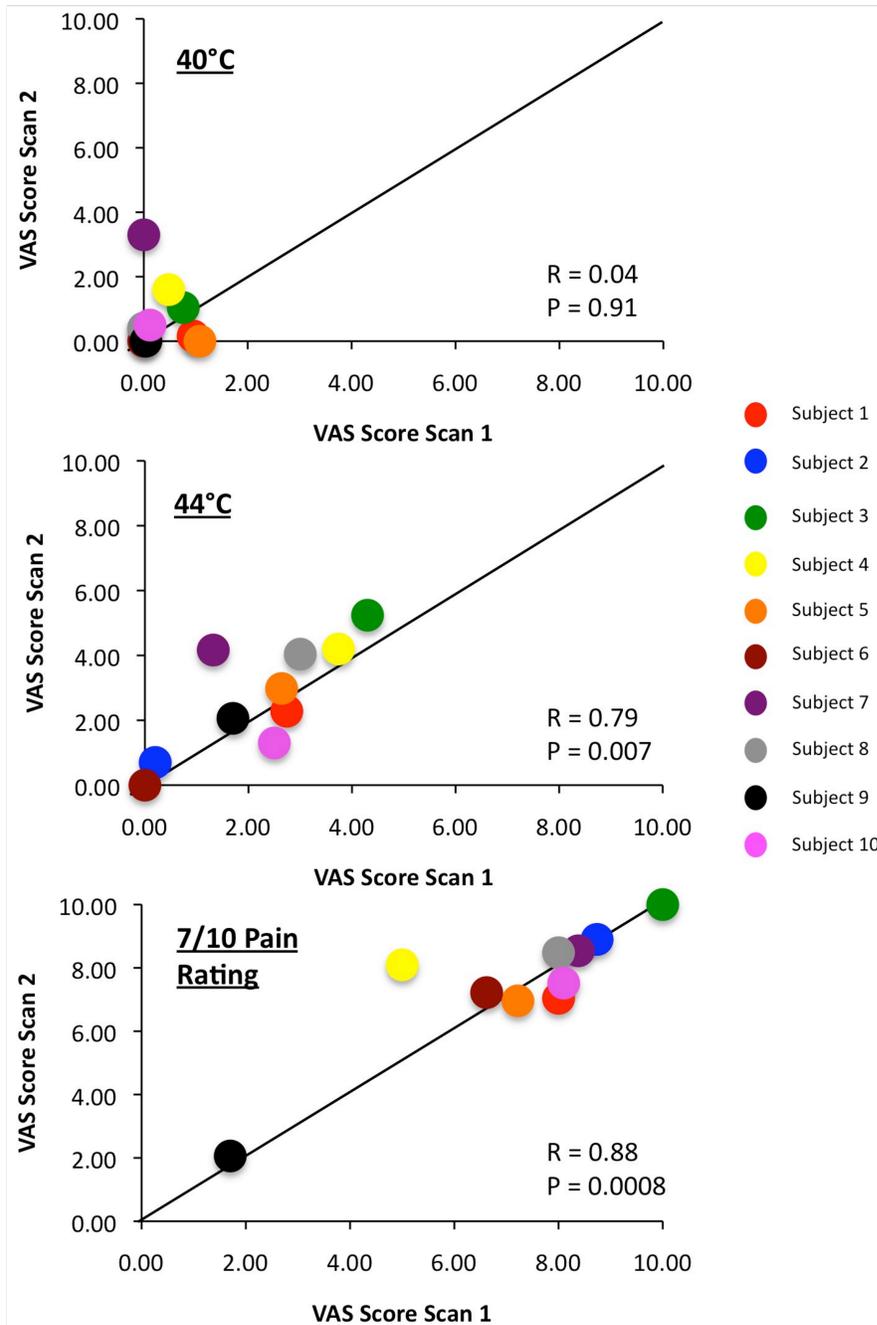
During fMRI experimentation, participants rated their subjective pain experience felt during each heat stimuli on a VAS pain scale (0 = no pain and 10 = maximal pain). The group-average pain ratings validated that 40°C, 44°C and 7/10 heat stimuli would respectively be non-noxious, mildly noxious and noxious (**Table 2**). A significant difference between the VAS ratings of pain intensity for the three temperatures was found ( $p < 0.05$ ; ANOVA). As expected, the 40°C stimulus and 7/10 heat stimuli were predominately reported as being non-noxious and noxious, respectively. Moreover, CIs at the 95% confidence level demonstrated similar magnitudes of lower and upper CI bounds across the three thermal stimulation types.

Test-retest reliability for VAS pain ratings for each temperature was obtained by calculating ICC values between scans 1 and 2 (**Table 2**). For the 44°C and 7/10 heat pain stimuli, ICC between scans 1 and 2 were high, while significant ICC was not observed for the 40°C. The latter was believed to result from little or no pain being perceived and reported by healthy subjects. The ICC stemming from the 7/10 subjective pain rating was the highest ICC value obtained in this study.

**Table 2: Summary of subjective reports obtained during 40°C, 44°C and 7/10 thermal pain stimulation paradigms.** VAS pain ratings are given in terms of mean  $\pm$  SE. CIs are reported at the 95% confidence level. \*Indicates no test-retest reliability between scans 1 and 2. SE – Standard Error; ICC – Intraclass Correlation Coefficient; CI – Confidence Interval

Stimulation	Scan Number	VAS Mean	ICC	F value	P-value	CI Lower	CI Upper
40°C	1	0.34 $\pm$ 0.4	-0.308*	0.762	0.654	-1.254	0.460
	2	0.74 $\pm$ 1.0					
44°C	1	2.21 $\pm$ 1.4	0.860	7.787	0.003	-1.240	0.275
	2	2.70 $\pm$ 1.7					
7/10	1	7.18 $\pm$ 2.3	0.938	15.694	0.001	-1.089	0.477
	2	7.48 $\pm$ 2.1					

Single-subject, VAS responses corresponding to 40°C, 44°C and 7/10 heat stimuli are shown in **Figure 1**. While no significant correlation in behavioral pain responses between scans 1 and 2 were observed, significant correlation and reproducibility in pain ratings were measured for both 44°C and 7/10 heat stimuli.



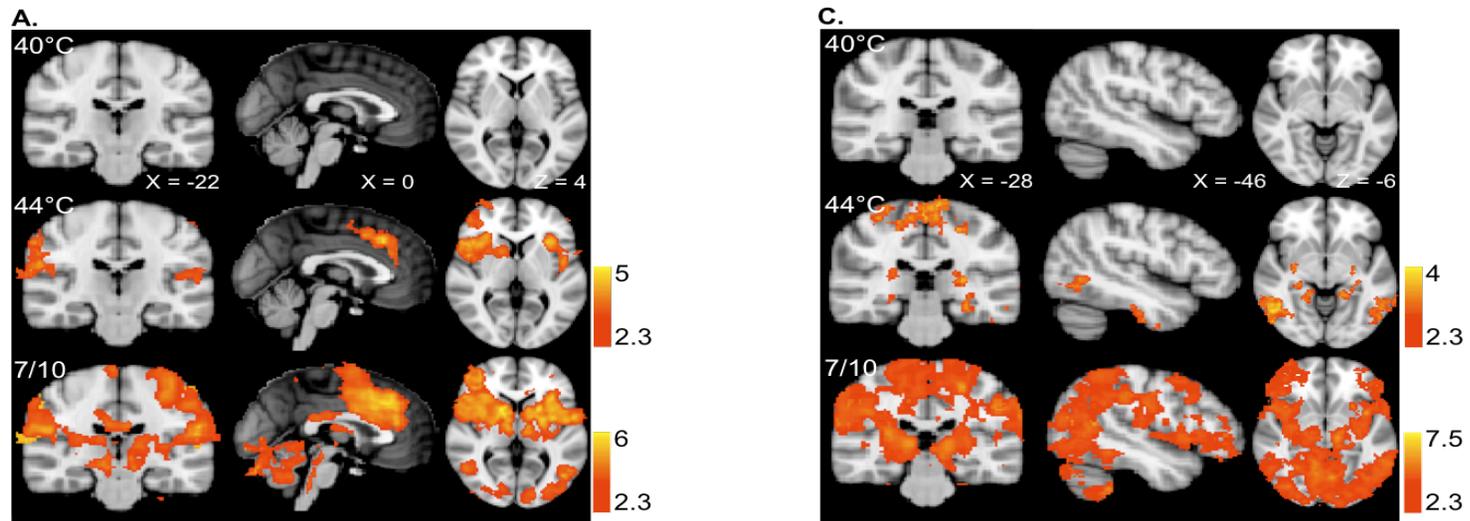
**Figure 1: Single Single-subject evoked VAS pain ratings.** Single-subject pain ratings for 40°C, 44°C and 7/10 pain stimuli for scans 1 and 2 are given in scatter plot format. Greater reproducibility at the single-subject level can be ascertained from data points projected closer to the centerline possessing a slope of 1.

### **QA of MRI Data**

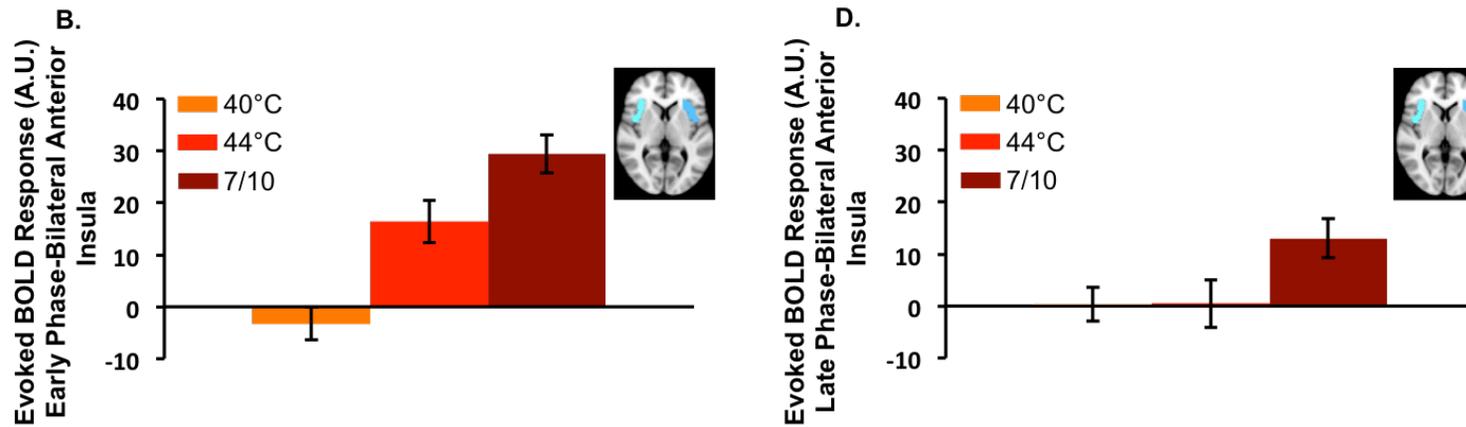
All fMRI data surpassed the fBIRN QA procedure. However, upon inspection of motion correction results, data from 2 of the 12 subjects was observed to have head motion greater than the in-plane resolution utilized during fMRI data acquisition. VAS pain rating and BOLD fMRI results provided and discussed correspond to an N=10 dataset.

### **Early and Late Phase Evoked BOLD Responses**

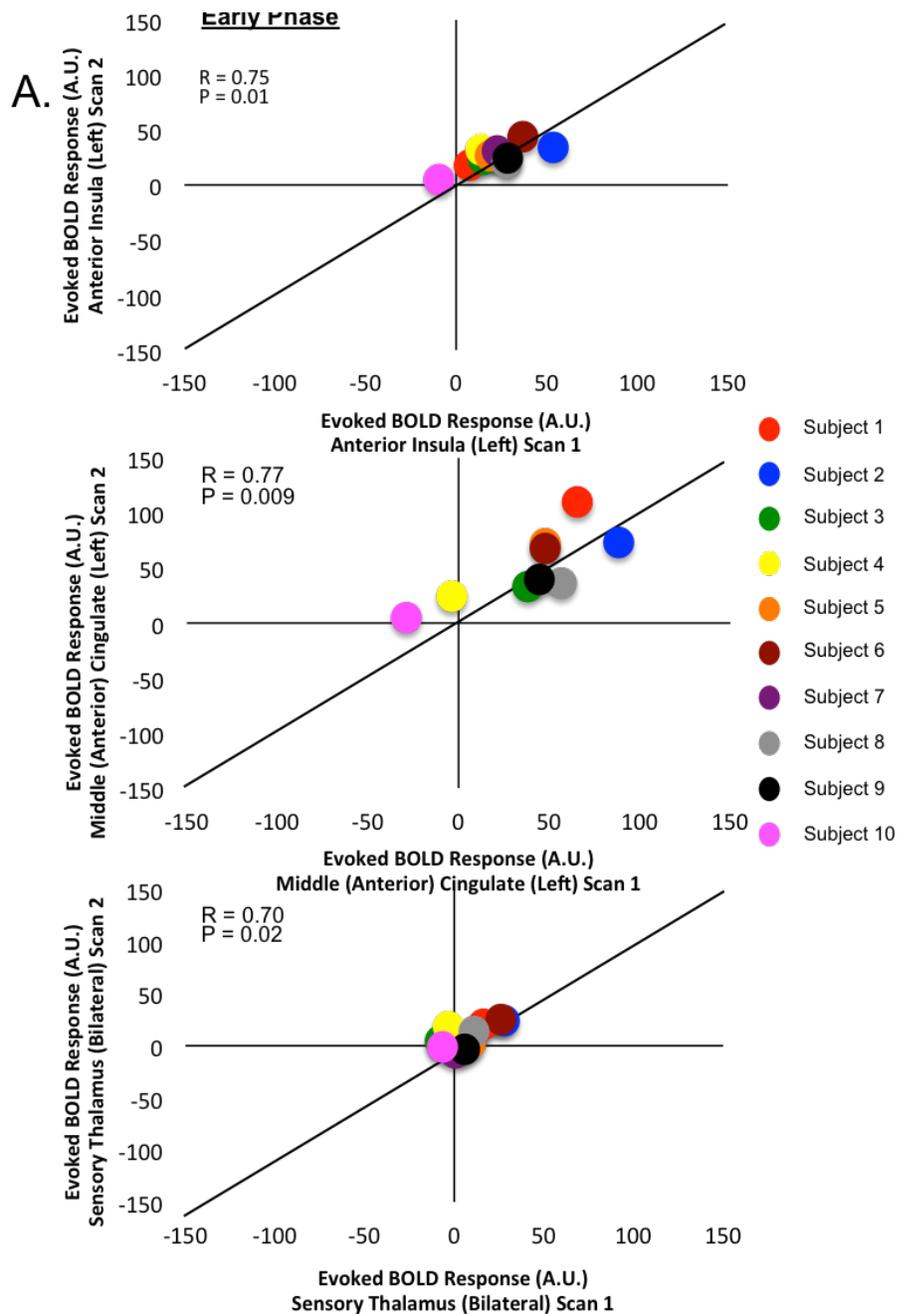
Similar to VAS pain rating data, statistical BOLD activation maps corresponding to early (**Figure 2A**) and late (**Figure 2C**) phase BOLD responses showed an overall pattern of increased activation when progressing from 40°C, 44°C and 7/10 heat stimuli. This progression was clearly observed in cortical (insula) and deeper subcortical (striatum) structures within the supraspinal pain processing pathways. To further elucidate the behavior of pain processing CNS structures during early and late phase evoked BOLD responses, parameter estimates from the anterior insula (bilateral, atlas defined) were extracted and averaged across subjects and scanning sessions. Interestingly, while a graded group average response was detected in the early phase within the anterior insula (**Figure 2B**), the anterior insula's late phase response (**Figure 2D**) there was no measurable response for the 40°C and 44°C stimuli and a significant BOLD response was detected for the 7/10 heat pain stimuli.



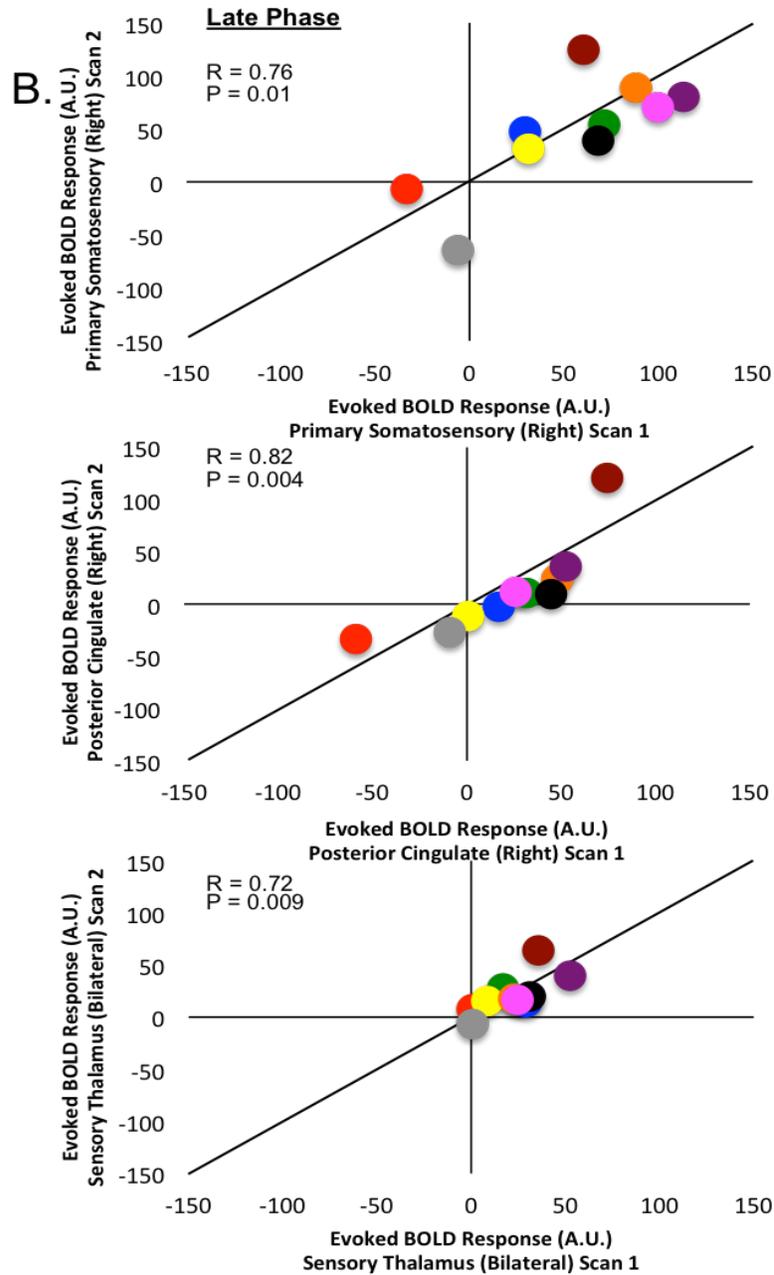
**Figure 2: Group-average evoked BOLD responses to 40°C, 44°C and 7/10 pain stimulation.** Within group average response were obtained by combining fMRI data across all subjects ( $n=10$ ) as well as across scan sessions 1 and 2. Statistical maps obtained from mixed-effects analyses ( $z > 2.3$  and cluster size corrected) are shown for each stimulation type and for early (A.) and late phase (B.) BOLD responses. Summary statistics extracted from the anterior insula show a graded response during the early phase (C.). The ANOVA test for the anterior insula, early phase BOLD responses revealed significant between condition differences ( $F_{2,57} = 19.88$ ,  $p = 2.88E-07$ ). Late phase BOLD responses in the anterior insula (D.) showed little or no response during the 40°C and 44°C stimulation, while the 7/10 pain stimulation was comparably more robust. The ANOVA test revealed significant between condition differences ( $F_{2,57} = 3.42$ ,  $p = 0.04$ ).



**Figure 2 continued: Group-average evoked BOLD responses to 40°C, 44°C and 7/10 pain stimulation.** Within group average response were obtained by combining fMRI data across all subjects ( $n=10$ ) as well as across scan sessions 1 and 2. Statistical maps obtained from mixed-effects analyses ( $z > 2.3$  and cluster size corrected) are shown for each stimulation type and for early (A.) and late phase (B.) BOLD responses. Summary statistics extracted from the anterior insula show a graded response during the early phase (C.). The ANOVA test for the anterior insula, early phase BOLD responses revealed significant between condition differences ( $F_{2,57} = 19.88$ ,  $p = 2.88E-07$ ). Late phase BOLD responses in the anterior insula (D.) showed little or no response during the 40°C and 44°C stimulation, while the 7/10 pain stimulation was comparably more robust. The ANOVA test revealed significant between condition differences ( $F_{2,57} = 3.42$ ,  $p = 0.04$ ).



**Figure 3: Single-subject evoked BOLD responses to 7/10 pain stimulation.** Single-subject early (A.) and late (B.) phase BOLD responses (defined by parameter estimates) for scans 1 and 2 are given in scatter plot format. Greater reproducibility at the single-subject level can be ascertained from data points projected closer to the centerline possessing a slope of 1. Parameter estimates were extracted from atlas defined cortical and subcortical ROIs. Corresponding ICC values for each ROI have been given in **Table 3C**.



**Figure 2 continued: Single-subject evoked BOLD responses to 7/10 pain stimulation.** Single-subject early (A.) and late (B.) phase BOLD responses (defined by parameter estimates) for scans 1 and 2 are given in scatter plot format. Greater reproducibility at the single-subject level can be ascertained from data points projected closer to the centerline possessing a slope of 1. Parameter estimates were extracted from atlas defined cortical and subcortical ROIs. Corresponding ICC values for each ROI have been given in **Table 3C**.

Single-subject, BOLD responses within cortical and subcortical ROIs as well as between scans 1 and 2 were evaluated for the 7/10 heat pain stimuli (**Figure 3**). Overall, across CNS structures and subjects good reliability can be visually observed with regards to the magnitude of the early (**Figure 3A**) and late (**Figure 3B**) phase BOLD responses. A comparison across the structures evaluated demonstrates higher consistency of BOLD responses between the two scan sessions for structures such as the anterior insula (early phase) and posterior cingulate (late phase) and lower consistency for the primary somatosensory cortex (late phase). To detect significant differences in early and late phase BOLD responses between scans 1 and 2, paired comparisons were performed for each of the three heat stimulation paradigms. The results of the paired, t-tests are summarized in **Tables 3-5**.

**Table 3: Regions with significant differences** (paired, t-test,  $z > 2.3$  and cluster size corrected) in early and late phase BOLD responses to 40°C heat stimulation between scans 1 and 2.

Brain Region	Lat.	z-stat	X(mm)	Y(mm)	Z(mm)	Vol(cm)
<b>Early Phase</b>						
<b>Frontal</b>						
Frontal Pole	R	3.4839	46	48	-22	0.648
Inferior Orbital	R	3.4545	36	40	-20	1.112
Inferior Triangular	L	3.7205	-54	18	24	0.752
Inferior Operculum	L	3.1722	-52	16	18	0.880
Precentral	L	2.9709	-60	0	26	0.424
Planum Polare	R	3.0661	42	-2	-18	0.560
<b>Parietal</b>						
Postcentral	L	2.8821	-56	0	14	0.376
	L	3.1526	-64	-8	22	0.472
	L	3.1302	-60	-8	30	0.552
	L	3.1912	-20	-42	50	0.400
<b>Occipital</b>						
Rolandic Operculum	R	3.1328	60	-2	16	0.640
	R	3.3575	60	-14	12	0.952
	R	2.9536	44	-30	18	0.488
Superior	R	2.8929	26	-70	32	0.456
<b>Temporal</b>						
Middle	R	3.4461	60	4	-20	0.856
	L	3.7127	-54	-32	4	0.472
	R	3.1961	64	-38	2	0.520
Temporal	L	2.8518	-34	2	-20	0.616
Superior	L	3.1074	-64	-14	10	0.480
	R	4.0583	62	-22	2	0.552
Inferior	R	3.3370	52	-32	-30	0.440
<b>Insula</b>						
Insular Cortex	L	3.2601	-38	-18	-6	0.528
<b>Late Phase</b>						
<b>Occipital</b>						
Calcarine	L	3.0880		2	-88	-8 0.400
<b>Brainstem / Cerebellum</b>						
Cerebellum Crust II	R	3.3060		36	-74	-40 0.872

**Table 4: Regions with significant differences** (paired, t-test,  $z > 2.3$  and cluster size corrected) in early and late phase BOLD responses to 44°C heat stimulation between scans 1 and 2.

Brain Region	Lat.	z-stat	X(mm)	Y(mm)	Z(mm)	Vol(cm)
<b>Early Phase</b>						
<b>Frontal</b>						
Superior	L	3.3892	-28	66	0	1.224
<b>Parietal</b>						
Inferior	L	3.5494	-50	-34	48	0.928
	R	3.1249	58	-54	42	0.456
<b>Late Phase</b>						
<b>Frontal</b>						
Precentral	L	3.6118	-22	-28	58	0.792
<b>Parietal</b>						
Precuneus	R	3.1607	6	-52	74	0.416
<b>Occipital</b>						
Middle	L	3.3052	-36	-72	4	0.672
Cuneus	R	3.4681	18	-74	36	0.728
	R	3.0509	10	-88	38	0.584
Lateral	L	3.3172	-50	-74	22	0.912
Superior	L	3.6403	-14	-80	42	4.08
	R	3.5776	26	-88	30	0.664
<b>Temporal</b>						
Lingual	L	3.473	-12	-58	-8	0.784
	L	3.5137	-12	-62	-10	0.48
<b>Brainstem / Cerebellum</b>						
Cerebellum VI	L	3.443	-16	-58	-16	0.528
	R	3.2323	16	-64	-14	0.408
	L	3.4642	-10	-68	-18	1.288
Vermis IV & V		3.3084	4	-60	-8	1.824

**Table 5: Regions with significant differences** (paired, t-test,  $z > 2.3$  and cluster size corrected) in early and late phase BOLD responses to 7/10 heat stimulation between scans 1 and 2.

<b>Brain Region</b>	<b>Lat.</b>	<b>z-stat</b>	<b>X(mm)</b>	<b>Y(mm)</b>	<b>Z(mm)</b>	<b>Vol(cm)</b>
<b>Early Phase</b>						
<b>Brainstem / Cerebellum</b>						
Cerebellum VI	L	3.2968	-24	-62	-22	0.824
<b>Late Phase</b>						
<b>Frontal</b>						
Superior	R	3.4929	4	62	16	0.520
	R	3.3079	14	62	8	0.560
	R	3.4968	6	60	24	0.432
	R	3.3122	8	56	32	0.376
	L	3.4071	-10	56	34	0.504
	R	3.4394	14	52	26	1.096
	L	3.2195	-18	44	36	1.312
	L	3.2103	-22	16	58	0.416
Superior Medial	L	3.5368	0	62	30	0.408
	L	3.3261	-14	62	10	0.616
	L	3.3021	-6	60	28	0.432
	Middle Orbital	R	3.4895	34	58	0
R		3.3259	28	4	50	0.464
Middle	L	3.2574	-28	58	16	0.696
	L	3.2254	-32	20	28	0.688
Superior Orbital	L	3.3075	-28	52	-4	0.488
	R	3.2654	20	30	42	0.416
	R	3.2616	20	18	54	0.384
	Frontal Pole	L	3.4894	-20	46	-8

**Table 5: Regions with significant differences** (paired, t-test,  $z > 2.3$  and cluster size corrected) in early and late phase BOLD responses to 7/10 heat stimulation between scans 1 and 2.

<b>Parietal</b>						
Postcentral	L	3.2773	-34	-22	50	0.896
SupraMarginal	R	3.5677	68	-42	24	0.376
Precuneus	R	3.3792	4	-54	26	1.016
	R	3.4292	8	-66	46	0.640
	R	3.2178	8	-70	50	0.600
<b>Occipital</b>						
Inferior	R	3.2428	42	-74	-16	0.416
	L	3.4305	-52	2	-36	0.640
	L	3.4108	-56	-28	-24	0.672
<b>Temporal</b>						
Middle	L	3.9546	-62	-4	-28	1.008
	L	3.2570	-62	-14	-6	0.408
<b>Cingulum</b>						
Anterior	R	3.2600	6	50	10	0.384
	L	3.2653	-2	34	6	0.584
Cingulate Gyrus	L	3.3282	-10	38	6	0.816
	L	3.2989	2	-40	22	0.752
Posterior	L	3.3007	-2	-50	26	0.632
<b>Brainstem / Cerebellum</b>						
Cerebellum IX	L	3.2448	-10	-48	-44	0.472
	R	3.3085	6	-54	-48	0.432
Vermis IX		3.3567	0	-52	-34	0.392
		3.7068	0	-62	-42	0.712

### ICC Values and CI for Evoked BOLD Responses

ICCs were quantified across all ROIs noted in **Table 1** and for the three heat stimulation conditions, 40°C, 44°C and 7/10 heat stimuli (**Table 3**). Very few CNS structures surpassed the  $p < 0.05$  threshold (corresponding to ICC of  $\sim 0.5$ ) for the 40°C (**Table 3A, 3B**) or 44°C (**Table 3C, 3D**) experimental conditions. Moreover, the ICC values for 40°C and 44°C stimuli ranged from 0.520 - 0.682. In sharp contrast, the 7/10 heat pain stimuli (**Table 3E, 3F**) yielded a substantially greater number of CNS structures surpassing the  $p < 0.05$  threshold (ICC range: 0.515 - 0.859) for both early and late phase BOLD responses.

**Table 6:** ICC values and CIs across ROIs possessing a p value of 0.05 and lower. ICCs and CIs were calculated for early and late phase evoked BOLD responses for 40°C (A. *Early Phase*, B. *Late Phase*.), 44°C (C. *Early Phase*, D. *Late Phase*.) and 7/10 (E. *Early Phase*, F. *Late Phase*.) thermal pain stimulation. CIs are reported at the 95% confidence level.

A. 40°C		Early Phase			
Regions of Interest	ICC	F-value	P-value	CI Lower	CI Upper
Thalamus (right)	0.643	4.119	0.023	-9.479	10.210

B. 40°C		Late Phase			
Regions of Interest	ICC	F-value	P-value	CI Lower	CI Upper
Superior Frontal (left)	0.575	5.606	0.009	1.590	14.252
Cerebellum VI (right)	0.615	4.298	0.020	-3.239	10.018
Cerebellum Crust I (right)	0.532	3.291	0.045	-5.427	14.514
Supplementary Motor Area (left)	0.580	3.715	0.032	-7.576	17.669
Sensory Thalamus	0.627	4.159	0.023	-3.802	6.721
Thalamus (right)	0.614	4.441	0.018	-3.071	12.037

**Table 6 continued:** ICC values and CIs across ROIs possessing a p value of 0.05 and lower. ICCs and CIs were calculated for early and late phase evoked BOLD responses for 40°C (A. *Early Phase*, B. *Late Phase*.), 44°C (C. *Early Phase*, D. *Late Phase*.) and 7/10 (E. *Early Phase*, F. *Late Phase*.) thermal pain stimulation. CIs are reported at the 95% confidence level.

C. 44°C		Early Phase			
Regions of Interest	ICC	F-value	P-value	CI Lower	CI Upper
Inferior Frontal Triangular (right)	0.552	3.252	0.047	-9.506	13.161
Inferior Frontal Opercularis (right)	0.682	4.885	0.014	-11.438	14.152
Anterior Insula (right)	0.620	4.092	0.024	-19.850	10.500

D. 44°C		Late Phase			
Regions of Interest	ICC	F-value	P-value	CI Lower	CI Upper
Cerebellum VIII (right)	0.536	3.213	0.049	-5.309	10.628
Cerebellum VIII (left)	0.546	3.296	0.045	-6.366	12.600
Cerebellum X (left)	0.563	3.398	0.041	-10.032	16.464

**Table 6 continued:** ICC values and CIs across ROIs possessing a p value of 0.05 and lower. ICCs and CIs were calculated for early and late phase evoked BOLD responses for 40°C (A. *Early Phase*, B. *Late Phase*.), 44°C (C. *Early Phase*, D. *Late Phase*.) and 7/10 (E. *Early Phase*, F. *Late Phase*.) thermal pain stimulation. CIs are reported at the 95% confidence level.

E. 7/10 VAS Pain Rating		Early Phase			
Region of Interest	ICC	F-value	P-value	CI Lower	CI Upper
Medial Superior Frontal (left)	0.556	3.282	0.046	-19.423	14.487
Medial Superior Frontal (right)	0.515	2.909	0.064	-13.836	14.654
Superior Frontal (left)	0.642	4.240	0.021	-10.093	11.270
Superior Frontal (right)	0.637	4.338	0.020	-10.696	5.535
Superior Frontal Orbitalis (left)	0.773	7.386	0.003	-17.986	10.261
Inferior Frontal Orbitalis (left)	0.787	7.796	0.003	-7.546	11.020
Inferior Frontal Orbitalis (right)	0.625	4.362	0.019	-11.203	4.176
Anterior Insula (left)	0.657	5.014	0.012	-12.674	3.834
Anterior Middle Cingulate (left)	0.748	7.490	0.003	-25.797	6.222
Anterior Middle Cingulate (right)	0.646	5.697	0.008	-28.793	1.971
Supplemental Motor Area (left)	0.585	3.656	0.033	-27.117	15.050
Supplemental Motor Area (right)	0.566	3.735	0.031	-29.396	8.858
Inferior Frontal Opercularis (right)	0.706	7.668	0.003	-21.464	0.252
Putamen (left)	0.568	3.402	0.041	-11.492	8.118
Sensory Thalamus (Bilateral)	0.715	5.661	0.008	-8.450	5.225
Posterior Middle Cingulate (left)	0.648	4.449	0.018	-15.646	9.053
Posterior Middle Cingulate (right)	0.695	5.644	0.008	-16.453	5.797
Posterior Insula (left)	0.715	5.805	0.008	-9.371	4.698
Posterior Insula (right)	0.551	3.313	0.045	-13.932	7.769
Hypothalamus (left)	0.489	3.273	0.046	-45.560	6.474
Thalamus (left)	0.637	4.378	0.019	-12.957	6.254
Thalamus (right)	0.726	7.430	0.003	-13.155	1.588
Cerebellum IV & V (right)	0.823	9.532	0.001	-9.282	6.568
Cerebellum IV & V (left)	0.675	4.958	0.013	-7.909	15.432
Cerebellum VI (left)	0.619	3.924	0.027	-17.981	17.207
Cerebellum VI (right)	0.578	3.472	0.039	-17.800	14.839
Cerebellum VIIb (left)	0.750	6.556	0.005	-14.648	9.181
Cerebellum VIIb (right)	0.644	4.294	0.020	-15.593	11.592
Cerebellum VIII (left)	0.701	5.221	0.011	-13.742	12.159
Cerebellum VIII (right)	0.708	5.366	0.010	-10.109	11.356

**Table 6 continued:** ICC values and CIs across ROIs possessing a p value of 0.05 and lower. ICCs and CIs were calculated for early and late phase evoked BOLD responses for 40°C (A. *Early Phase*, B. *Late Phase*), 44°C (C. *Early Phase*, D. *Late Phase*.) and 7/10 (E. *Early Phase*, F. *Late Phase*.) thermal pain stimulation. CIs are reported at the 95% confidence level.

F. 7/10 VAS Pain Rating	Late Phase				
Region of Interest	ICC	F-value	P-value	CI Lower	CI Upper
Amygdala (left)	0.724	5.796	0.008	-18.169	24.764
Sensory Thalamus (Bilateral)	0.731	5.906	0.007	-9.158	10.161
Posterior Middle Cingulate (left)	0.559	3.419	0.041	-12.348	24.559
Posterior Insula (left)	0.665	4.618	0.016	-9.478	12.579
Thalamus (left)	0.633	4.163	0.023	-12.246	18.051
Thalamus (right)	0.649	4.413	0.019	-16.370	10.784
Primary Somatosensory Cortex (right)	0.761	6.908	0.004	-19.523	31.063
Posterior Cingulate (left)	0.623	5.019	0.012	-4.245	34.890
Posterior Cingulate (right)	0.812	9.813	0.001	-9.051	26.390
Cerebellum III (left)	0.587	3.568	0.036	-12.501	14.971
Cerebellum IV & V (left)	0.744	6.302	0.006	-13.191	9.722
Cerebellum IV & V (right)	0.759	6.712	0.005	-10.097	8.386
Cerebellum VI (left)	0.816	9.440	0.001	-18.293	9.608
Cerebellum VI (right)	0.859	11.973	0.001	-10.926	10.592
Cerebellum VIIb (left)	0.703	5.407	0.010	-14.726	9.057
Cerebellum VIII (left)	0.835	10.207	0.001	-6.938	9.104
Cerebellum VIII (right)	0.678	4.818	0.014	-9.773	12.435
Cerebellum IX (left)	0.587	5.563	0.009	1.894	23.381
Cerebellum IX (right)	0.678	4.818	0.014	-3.145	30.200
Cerebellum X (right)	0.564	3.605	0.035	-11.967	32.231
Cerebellum Crust I (left)	0.645	4.323	0.020	-24.706	17.437
Cerebellum Crust I (right)	0.757	6.637	0.005	-17.112	19.650

When evaluating CIs at the 95% confidence level in relation to the corresponding ICC value, a relationship between the magnitudes of upper or lower CI bounds with ICC values was not observed. Based on CIs, low variance was observed for early phase BOLD responses in structures such as the anterior and posterior insula, putamen and thalamus (including sensory thalamus), while higher variability was calculated for late phase BOLD responses such as the posterior cingulate and primary somatosensory cortex. However, the magnitudes of CIs (lower and upper bounds) for early

## DISCUSSION

### Summary of Findings

The current study was design to reflect a standard randomized clinical trial and crossover design with drug and placebo. The study assessed the graded, group-level response and importantly, the test-retest reliability of VAS pain ratings and CNS BOLD responses measured during administration of three, evoked thermal stimulations; 40°C, 44°C and a subject-specific temperature eliciting a subjective pain level of 7/10. Based on ICC values calculated between two scanning sessions and across study endpoints, reproducibility was observed to be highest for VAS pain ratings obtained during the 7/10 pain stimulation (ICC = 0.938). This could change significantly once similar work is carried out in patient populations rather than in healthy subjects.

With respect to test-retest reliability of pain fMRI data, the 7/10 pain stimuli also yielded the greatest number of CNS structures possessing ICC levels of  $\sim 0.5$  or more ( $p < 0.05$ ; ICC range: 0.5 - 0.859). For all VAS pain ratings and BOLD fMRI data points surpassing the threshold of  $p < 0.05$ /ICC of  $\sim 0.5$ , CIs were also calculated. In future trials where an analgesic might be evaluated in healthy male subjects; the upper and lower CI bounds could be utilized to determine the magnitude of therapeutic effect necessary to overcome inherent variability of

either VAS pain ratings or BOLD responses evoked during innocuous or noxious thermal stimulation.

### **Comparison of Pain fMRI Test-Retest Results**

Similar to previous investigations measuring test-retest reliability of pain fMRI measures [9; 14; 18], ICC values of BOLD responses evoked during noxious thermal stimulation were calculated across CNS structures. A common theme amongst studies was the fact that high ICC values within BOLD fMRI data collected during noxious conditions were observed in sub-regions within the cingulate, insular and frontal cortices. For example, for the anterior middle cingulate cortex, the current study, Letzen et al. and Quinton et al. all observed ICCs between ~0.7 and ~0.8 [9; 14]. Further congruence in terms of ICCs for BOLD fMRI data may have been present between this and past studies; however, in earlier work, the CNS ROIs evaluated did not include regions such as the amygdala, striatum or cerebellum. One notable difference between the work by Quinton et al., and this study pertains to the reproducibility of BOLD responses of ascending pain pathway structures (i.e., sensory thalamus and primary somatosensory cortex) during noxious stimulation. While the earlier study found insignificant or low test-retest reliability within the thalamus or primary somatosensory cortex, we observed that early and late phase BOLD responses possessed ICC values of similar magnitude (0.7-0.75) to cingulate, insular and frontal cortices. This disparity may arise as a result of

methodological differences such as how these ascending pain pathway structures were anatomically defined or statistical modeling procedures of BOLD fMRI data (See Below). Moreover, in accord with Letzen et al., we observed higher intersession reliability for subjective pain ratings compared to evoked BOLD responses in general.

The evaluation of VAS pain ratings and BOLD responses for a range of thermal stimuli of increasing pain intensity enabled a graded CNS BOLD response to be detected, but importantly, demonstrated that insignificant or low test-retest reliability was present for innocuous or mildly noxious thermal stimuli (i.e., 40°C and 44°C) compared to the 7/10 pain stimulus. It cannot be simply concluded that compared to low, non-noxious temperatures, higher temperatures will conventionally yield higher reproducibility of BOLD fMRI data. It is likely that while some individuals can withstand, for example, a 50°C stimuli, others may find this temperature too intense, possess head motion and subsequently introduce variability into the data. Thus, as performed in this and earlier work, to obtain significant test-retest reliability of VAS pain ratings and BOLD fMRI data, it is necessary to have a balance between a noxious stimuli as defined by the subject or patient, which is intense enough to remain noxious between 2 or more scanning sessions, yet not noxious to the point where study volunteers may find the stimulation paradigm unbearable.

It is noted that comparing reproducibility results across pain fMRI investigations was not necessarily a straightforward process given methodological differences each study incorporated in terms of evoked pain stimulation paradigms, data acquisition and data analyses. For example, the evoked thermal pain stimuli utilized by Quinton et al., were 48°C and a subject-specific heat stimuli yielding a 50/100 VAS pain rating, while Letzen et al. used a ‘tolerated’ temperature between 43 and 51°C. This is in contrast to the 7/10 subject-specific temperature utilized herein. In the reports by Letzen et al. as well as Quinton et al. reliability of BOLD responses of early and late phase BOLD responses throughout the CNS were not assessed. Given the differences of the explanatory variables used in GLM analyses of BOLD fMRI data, between-study differences in ICC values for a single CNS structure could arise strictly as a result of the analytical approach taken. Nonetheless, when considering the reproducibility results reported across studies and in pain processing CNS structures (i.e., cingulate, insular and frontal cortices) what can be said with confidence is that sufficient intersession reliability exist for pain fMRI, such that the method can be implemented to evaluate CNS pain processing in longitudinal studies involving healthy subjects. The utility of pain fMRI is further warranted when considering the work of Wager and colleagues, where an fMRI-based signature of pain demonstrated high sensitivity and specificity as well as treatment response [23]. Interestingly, the fMRI signature of pain identified in the latter study consisted of

CNS structures such as the thalamus, insula and cingulate; structures that showed significant reproducibility in the past and current fMRI investigation.

### **Study Limitations**

#### ***Number of Scan Sessions:***

Intersession reliability of behavioral and fMRI data may vary based on the number of scan sessions used to assess methodological test-retest reliability. Previous studies have used a comparison of three scan sessions to determine reproducibility [9; 14]. In contrast, the design implemented in the current work was setup with a placebo-controlled, cross over study paradigm in mind, given its utility in trials evaluating pharmacological therapies. Nonetheless, the inclusion of additional scanning sessions or a longer intersession time interval may enable a better evaluation of reliability and stability of behavioral and fMRI measures.

#### ***Subjects:***

Compared to earlier trials investigating test-retest reliability of evoked pain fMRI, the cohort size of the present study was considered small (n=10). The univariate analysis results for BOLD fMRI data did indeed elicit the expected robust response within the supraspinal CNS structures mediated pain, particularly for the 7/10 pain stimulation. However, with a larger study population, a more accurate account of variance for both pain ratings and BOLD fMRI data may be achieved.

The primary goal of this study was to evaluate the reproducibility of evoked pain methodology itself, and therefore, a 'homogeneous' population of healthy male subjects was enrolled. Given the known differences in pain processing between males and females, the projection of the current results to a female population or a broader population encompassing both males and females should be done with caution [12]

***Habituation:***

As with most fMRI studies, including those investigating evoked pain responses, stimuli are often repeatedly presented in order to increase the SNR (signal to noise ratio) of the BOLD signal. This repetition could induce a habituation effect, and in turn introduce variance into the data. Here, each stimulus (40°C, 44°C and 7/10 heat stimuli) was repeated 5 times, but in a randomized manner. The randomization is believed to have minimized the risk of habituation to any one particular stimulus as evidenced by the graded responses measured across the three stimuli as well as the group-average pain rating of ~7 obtained for the 7/10 pain stimulus. However, a complete negation of habituation would be difficult.

## **Future Directions**

A key step to further understanding the benefits and limitations of pain fMRI measures is to utilize the technique in a longitudinal manner in a pain patient population. In patients, fluctuations in disease specific pain, from magnitude, location and frequency perspectives, as well as other related disease factors (e.g., fatigue, depression or frequency of medications consumed) can be present across time. To what extent these patient-specific fluctuations impact the reliability and reproducibility of measures obtained in an fMRI study are in large part unknown. Moreover, by implementing pain fMRI longitudinally in patients, if and how fMRI measures track with core symptoms, which are of key clinical interest during therapeutic evaluation, may also be quantitatively understood. It may be the case that the natural waxing and waning of endogenous pain levels or other symptoms may introduce variability and thus lessen intersession test-retest reliability of evoked pain endpoints. However, it may be of value to elucidate the underlying pathophysiology of pain or symptom fluctuation in patients, as the former could be easier to target within the translational medicine setting.

In patients, much is unknown regarding how much potentiation or attenuation of the BOLD response measured in a CNS structure or network is necessary to overcome the inherent variance as well as to induce a meaningful clinical effect. By longitudinally measuring the pharmacodynamics of a standard of care for a

specific pain patient population, the magnitude of effect and its clinical relevance can be better realized. Given the diverse functional, structural and neurochemical changes that can be present between distinct pain conditions, it is likely that a single answer does not exist in terms how and what CNS brain region(s) should be therapeutically targeted.

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## CURRICULUM VITAE

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**Augustana College**      **Sioux Falls, South Dakota**      09/2009-05/2012  
Bachelor of Arts      Major: American Chemical Society Biological Chemistry

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### EMPLOYMENT

**Boston Children's Hospital**      Boston, Massachusetts  
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Studying functional MRI of chronic pain in pediatric patients.  
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• Phlebotomy  
• Obtaining EKGs  
• Room clean-up, Transporting patients, Assisting with equipment retrieval  
• Maintenance and quality control  
• Routine housekeeping and supply management

**Monitor Technician**  
• Paging physicians on call  
• Calling overhead traumas  
• Computer keyboarding skills  
• Monitoring and interpreting patient rhythms  
• Tearing down and assembling patient charts  
• Calling other departments to inform them of ordered tests and procedures  
• Monitoring security cameras within the emergency department

- Monitoring security access to emergency department

**Service Representative**

- Maintain a calm and caring affect even when crisis, chaos and/or tragedy may be occurring within the department
- Offer personal assistance and resource availability to patients, families, visitors or others.
- Responsible for the coordination of directing patients, family members and visitors to various departments and offices on the Sanford Health Hospital campus

**Health Management Partners**

Sioux Falls, South Dakota

Contact: Amber Watcher (605) 333- 0123

Health Screen Technician,

(11/2012-12/2012)

- These consist of a finger stick and then running a lipid panel and blood sugar
- Measure height, weight, waist circumference, and blood pressure
- At the end of each appointment I do some health coaching and make referrals as necessary
- Screenings in Rapid City, Pine Ridge, Watertown, Sioux Falls, Brookings, Vermillion, Mobridge, and more

**Sanford Hospital**

Post Anesthesia Care Unit Assistant,

(03/2010-11/2012)

Sioux Falls, South Dakota

Contact: Rose Ligtenberg (605) 328-2050

- Assist clinicians and physicians in procedures (central line placement, nerve blocks, and epidurals)
- Access to medications for purposes of distribution to licensed personnel
- Manage the front desk disturbing patients throughout the unit
- Answering phone calls and directing the calls to the correct person
- Transport patients to other areas of the hospital
- Wake patients up and survey critical patients
- Utilize appropriate age related patient protocols relating to the physical and psychological needs of patients
- Routine housekeeping and supply management

**Augustana College**

Biology Teaching Assistant,

Contact: Dr. Steven Matzner (605) 274-4700

Sioux Falls, South Dakota

(01/2012- 05/2012)

- Biology 121- Biological Principles II
- Set up lab equipment
- Assisted students with activities and answered questions
- Graded lab reports, lecture tests, and student presentations

**Alaska Clinical Research Center**

Clinical Research Technician,

Anchorage, Alaska

(06/2009-09/2009)

Contact: Robin Adams (907) 276-2803

- Assisted clinicians
- Read journal articles on current clinical studies
- Processed post labs
- Packaged and shipped out blood draws
- Researched for new studies
- Requested X-rays and MRI from other facilities
- Made spread sheets for data input
- Organized the supplies from the sponsoring drug company

### **VOLUNTEERING**

#### **Sertoma Butterfly House and Marine Cove**

**Sioux Falls, South Dakota**

(09/2012-12/2012)

- In charge of the marine touch pool
- Provide instructions to visitors how to touch sting rays and sharks
- Clean the tank and pick up any trash left by visitors
- Listen to the head marine biologist of aquarium for closing instructions
- Answer questions that visitors may have
- 20+ hours of volunteering

#### **Children of the Nations International Mission Trip Barahona, Dominican Republic**

Surgical Mission Trip (4/12/2013-3/20/2013 1/14/2012-1/22/2012)

#### **Avera Hospital**

**Sioux Falls, South Dakota**

Emergency Room

(09/2008- 03/2010)

- Communicate with clinicians and physicians
- Provided assistance in procedures
- Cleaned rooms, Transfer patients to areas of the hospital
- 72+ hours of volunteering

### **AWARDS**

Service Excellence Sanford Hospital 09/10/2012 and 12/28/2012

Tri-Beta National Biological Honor Society Augustana College 03/2010

Accepted into the Alpha Lambda Delta Honor Society South Dakota State Univ.  
03/2009