Optimizing and Interpreting Insular Functional Connectivity Maps Obtained During Acute Experimental Pain: The Effects of Global Signal and Task Paradigm Regression

James W. Ibrinson,¹ Keith M. Vogt,² Kevin B. Taylor,³ Shiv B. Dua,⁴ Christopher J. Becker,³ Marco Loggia,⁵ and Ajay D. Wasan¹

Abstract

The insula is uniquely located between the temporal and parietal cortices, making it anatomically well-positioned to act as an integrating center between the sensory and affective domains for the processing of painful stimulation. This can be studied through resting-state functional connectivity (fcMRI) imaging; however, the lack of a clear methodology for the analysis of fcMRI complicates the interpretation of these data during acute pain. Detected connectivity changes may reflect actual alterations in low-frequency synchronous neuronal activity related to pain, may be due to changes in global cerebral blood flow or the superimposed task-induced neuronal activity. The primary goal of this study was to investigate the effects of global signal regression (GSR) and task paradigm regression (TPR) on the changes in functional connectivity of the left (contralateral) insula in healthy subjects at rest and during acute painful electric nerve stimulation of the right hand. The use of GSR reduced the size and statistical significance of connectivity clusters and created negative correlation coefficients for some connectivity clusters. TPR with cyclic stimulation gave task versus rest connectivity differences similar to those with a constant task, suggesting that analysis which includes TPR is more accurately reflective of low-frequency neuronal activity. Both GSR and TPR have been inconsistently applied to fcMRI analysis. Based on these results, investigators need to consider the impact GSR and TPR have on connectivity during task performance when attempting to synthesize the literature.

Key words: blood oxygen level-dependent signal; electric nerve stimulation; functional connectivity MRI; global signal regression; pain; task paradigm regression

Introduction

IT IS WELL KNOWN that the perception and processing of pain do not activate a single region of the brain; instead, multiple overlapping networks are involved and often loosely termed as the “pain matrix” (Apkarian et al., 2005; Peyron et al., 2000). While these overlapping networks are activated by a range of salient stimuli (Legrain et al., 2011), they are typically divided into sensory processing components that include the primary (S1) and secondary (S2) somatosensory cortices and affective processing components focusing on the anterior cingulate (ACC), amygdala, and medial prefrontal cortices (Brooks and Tracey, 2005). The insula, however,

is uniquely located between the temporal and parietal cortices, making it anatomically well-positioned to act as an integrating center between the sensory and affective domains (Wiech et al., 2014). Furthermore, connectivity between the insula and other structures in the pain matrix has been described (Peltz et al., 2011).

To probe insular connections and interactions, investigators have turned to functional connectivity magnetic resonance imaging (fcMRI). This technique analyzes the covariation of low-frequency (<0.1 Hz) oscillations in the MRI signal between brain areas. These occur in an organized and coherent manner across brain areas that are functionally related (Fox and Raichle, 2007), likely reflecting the spontaneous neuronal

¹Department of Anesthesiology, Center for Pain Research, University of Pittsburgh, Pittsburgh, Pennsylvania.

²Department of Anesthesiology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

³University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

⁴George Washington University School of Medicine and Health Sciences, Washington, District of Columbia.

⁵Department of Radiology, Massachusetts General Hospital, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts.

activity, and are present at rest. This simplifies the experimental design—subjects only need to lie quietly in the scanner while the images are collected to determine resting-state functional connectivity. As such, resting-state fMRI methodologies can more readily be used to study ongoing and chronic physiological states, such as chronic pain. However, the resulting descriptions of the function of the insula in such patients contain inconsistencies. For example, the insula (among other areas) had an increased connectivity to an organized set of areas known as the default mode network (DMN) at baseline in patients with chronic low back pain (Kornelsen et al., 2013; Loggia et al., 2013) and in fibromyalgia (Napadow et al., 2010), whereas others found that insula to DMN connectivity at baseline was unchanged in complex regional pain syndrome (Bolwerk et al., 2013).

Functional connectivity (FC) during acute pain processing has been studied as well. Ichesco and colleagues (2012) showed that patients with pain due to the temporomandibular disorder had an increased connectivity between the insula and the ACC, both at rest and during an experimental heat pain task when compared to normal controls, but also showed that patients with higher clinical pain had less insula to ACC connectivity. In pediatric patients with complex regional pain syndrome, painful cold stimulation of the affected limb showed a general pattern of increased functional connectivity between multiple seed regions and the rest of the brain; however, there were no differences in insula connectivity during stimulation of the affected limb when compared to the unaffected limb (Linnman et al., 2013).

Part of the issue with studies investigating insular connectivity is that the insula appears to be divided into distinct subdivisions with different resting-state functional connectivities (Taylor et al., 2009) responsible for different aspects of pain processing (Wiech et al., 2014). Further complicating the interpretation of the above studies is the lack of a clear methodology for the analysis of FC. This is especially true during task performance, where detected connectivity changes may reflect actual alterations in the synaptic/nerve activity related to pain, may be due to changes in global cerebral blood flow, or may be due to the superimposed task-induced neuronal activity. Furthermore, each of the above studies varied considerably in their approach to preprocessing and connectivity analysis, especially with regard to two issues: global signal regression (GSR) and task-induced MRI signal changes. In GSR, a time course consisting of the MRI signal averaged across each 3D brain volume is used as a regressor of no interest. By doing so, GSR increases the contrast-to-noise ratio of the fMRI signal at the expense of introducing anticorrelation into the data (Fox et al., 2009; Van Dijk et al., 2010). These anticorrelations may complicate the interpretation of comparisons between the resting state and active task fMRI data, as areas that did not appear to be correlated in either initial condition could be significant in a difference map. Likewise, the superposition of task-induced MRI signal changes on top of the connectivity-related spontaneous oscillations in the task-activated areas (Fox and Raichle, 2007) may not be fully corrected by low-pass filtering, corrupting the connectivity changes that may occur during task performance. Task paradigm regression (TPR) has been suggested as a means of correcting this; assuming linear superposition of the task and connectivity signals suggests that removing the task-in-

duced changes through regression could then allow for more precise identification of the connectivity signal.

The primary goal of this study was to investigate the effects of GSR and TPR on the changes in functional connectivity of the insula in healthy subjects at rest and during acute pain stimulation. We hypothesized that GSR would modify statistical connectivity maps in two ways: decreased strength and extent of areas showing positive correlation to the seed region and the emergence of areas with negative seed correlation. In addition, the effectiveness of low-pass filtering at eliminating task-induced signal changes was assessed because TPR would only be necessary if low-pass filtering alone was insufficient. We hypothesized that TPR would make the pain versus rest connectivity difference calculated during a cyclic pain task more closely resemble the maps obtained during sustained pain. Experimental pain stimulation in healthy volunteers was used, as a recent review suggests that different chronic pain conditions show unique connectivity patterns (Apkarian et al., 2011) and this could confound our results.

Methods

Subjects

This study's protocol was approved by the Institutional Review Board of the University of Pittsburgh and complies with all relevant recommendations for responsible research. Fifteen healthy right-handed adult subjects (11 male) between the age of 18 and 50 were enrolled in this study. Subjects were screened for any contraindications to MRI as well as exclusion criteria that included current pregnancy or prescription medication use, history of current acute or chronic pain, and history of illicit substance use. An investigation of the temporal dynamics of the MRI signal using a portion of the data collected for this study has already been described (Ibinson and Vogt, 2013).

Painful stimulus paradigm

Transcutaneous electric nerve stimulation (ENS) was used as the experimental pain stimulus, as described previously (Ibinson and Vogt, 2013). Briefly, electrodes were placed on the lateral aspect of the subject's right index finger straddling the proximal interphalangeal joint. This location allowed for sensory fiber stimulation without the movement associated with motor fiber activation. The nerve stimulator (EzStim II; Life Tech, Stafford, TX) was connected to the electrodes through twisted-pair shielded lead wires that passed through an RF-filtering penetration panel into the scanner room. A 100 Hz stimulation waveform was used (tetanic setting on the nerve stimulator), and the current flow was individualized for each subject to a subjective pain intensity rating of 7/10. The numerical pain scale was explained to subjects as ranging from 0 to 10, with anchors of 0 being no pain and 10 as highest pain imaginable. Once adjusted, this current level (average $\pm$ standard deviation of 19 ± 12 mA for all subjects included in the final analysis) was used continuously throughout all pain stimulation periods as described below; each individual's amplitude and frequency were not varied once stimulation began.

The experiment involved two periods, with imaging occurring continuously throughout the experiment. Scanning

began with a 3.5-min resting-state scan, the data from which is labeled rest throughout the remainder of the article. Next came the stimulation period, referred to as cyclic pain, which involved alternating between 30-sec periods of painful ENS and 30 sec of rest in the block-design on/off pattern typical of task-based blood oxygen level-dependent (BOLD) functional MRI (fMRI) experiments. Four pain periods were included in cyclic pain for a total of 4.5 min and an overall experiment time of 8 min. The order of rest and cyclic pain was not counterbalanced across subjects to prevent effects from pain from lingering into the rest period.

As described below, the TPR comparison required the introduction of a tonic pain task that consisted of a single, continuous painful stimulation 2 min in duration. The order of delivery of the cyclic and tonic pain tasks was not randomized, however, they were separated by a 4-min rest period, which has previously been shown to prevent any confounding/carryover effects (Ibinson et al., 2004).

Scanning protocol

Imaging was performed using a Siemens 3.0 T Trio Scanner (Siemens Medical Solutions, Malvern, PA). After positioning in the scanner, a high-resolution T1-weighted image was acquired using a 3D magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence with the following parameters: TR (repetition time) 25 sec, TE (echo time) 5 ms, flip angle 35°, matrix size 256 × 192, field of view 20 cm, in-plane resolution 1.2 mm, and slice thickness 2.8 mm, contiguous. The fMRI data were acquired with a BOLD-weighted gradient echo sequence with the following parameters: TR 2 sec, TE 30 msec, flip angle 90°, matrix 64 × 64, in-plane resolution 3.125 × 3.125 mm, and slice thickness 4 mm. Thirty-five contiguous axial slices were collected in an interleaved manner, providing whole brain coverage. A total of 105 brain volumes for rest and 135 volumes for cyclic pain were collected and analyzed. All fMRI data sets were analyzed using FSL (www.fmrib.ox.ac.uk/fsl) version 4.1.8; image analysis acronyms refer to subroutines within FSL, unless otherwise specified.

GSR analysis

For the analysis of GSR, the 4D nonbrain extraction [using BET (Smith, 2002)] was immediately followed by the determination of the global signal (calculated as the mean of all non-zero voxels in each image volume). Motion correction was then conducted with MCFLIRT (Jenkinson et al., 2002). This step was followed by spatial smoothing using a Gaussian kernel of full-width at half maximum of 6 mm and low-pass filtering using a Gaussian filter with a sigma of 2.8 sec.

The signal from the posterior portion of left insula (contralateral to the stimulus) was extracted for use as the functional connectivity seed region. This was identified based on the location of activation detected in a previously reported task-based analysis of the cyclic pain data (Ibinson and Vogt, 2013). The center of mass of this activation cluster in the Montreal Neurological Institute (MNI) standard space brain (voxel coordinates = 65, 62, 42, with zeroed image origin) was used as the center for a 6 mm radius spherical region of interest (ROI). This posterior insula ROI mask was transformed and back-projected into the lower resolution (native) coordinate space of the individual subject's functional images

using the registration matrices from FLIRT, and the average ROI signal intensity time courses for each subject were then extracted for use as the seed time course.

After FILM prewhitening was applied, two models were then tested using the FEAT version 5.98: (i) the insula time course as the main effect of interest with the six motion parameters and the global mean signal added as effects of no interest (the with GSR model) and (ii) a model that did not include the global mean signal (without GSR). Comparisons between these models demonstrate the effect of GSR. Although GSR models with and without TPR were initially tested, both models here used TPR since results presented below suggest that TPR use is needed.

TPR analysis

Similar steps were used for the analysis of the effect of TPR on the pain connectivity maps. Brain extraction, motion correction, spatial smoothing, global signal extraction, and insula time course extraction were carried out as described above. Two additional models were then tested after prewhitening: (iii) the only TPR model using the insula time course as the main effect of interest with the six motion parameters, the global mean signal, and the task paradigm (convolved with a gamma hemodynamic response function) as effects of no interest in data that were not low-pass filtered and (iv) the Low Pass + TPR model that included low-pass filtering as defined above in the preprocessing in addition to the other effects of no interest.

Comparison of the above two models (iii and iv above) would isolate the effect of low-pass filtering on TPR; however, information on the appropriateness of this step required the use of an additional pain task with no task-related signal change. The tonic pain task described above was used to provide an active condition that would not have the abrupt signal changes in activate areas expected from a cyclic task.

Group functional map analyses

For all group analyses, individual subject's BOLD functional images were registered first to their high-resolution structural image and then to the MNI standard brain using linear registration with MCFLIRT. A paired *t*-test analysis was used to determine differences between the rest and cyclic pain data sets. Due to the relatively small sample size and the inherent variability in pain-induced brain activation (Mayhew et al., 2013), a fixed-effects model was used. Significant results are displayed with cluster thresholding at $Z > 4$ with a significance of $p = 0.0001$ (Worsley, 2001), except as otherwise noted.

Results

The rotational and translational motion reports from registration in MCFLIRT were examined, and it was noted that Subject 1 moved over 1 mm in a stimulus-correlated manner (further analysis showed that this subject had activation maps that could not be interpreted). As a result, this subject was excluded from further analysis. None of the remaining subjects showed excessive or stimulus-correlated motion, nor had other issues with data quality.

FIG. 1. Selected slices showing group average connectivity to a left insula seed region in the cyclic pain data set without the use of global signal regression (GSR). Columns 1 and 2 demonstrate the maps associated with typical image rendering settings ($Z > 4$ with color representing values from 0 to 15). Column 3 demonstrates that higher thresholds result in more discrete areas of connectivity. Comparisons of rest versus pain in columns 4, 5, and 6 show that pain resulted in increased connectivity across the brain, but individual areas are only identifiable with thresholding at $Z > 10$. Color images available online at www.liebertpub.com/brain

Effect of GSR

Without GSR, both the rest and the cyclic pain maps (Fig. 1) contain large areas of significant correlation when using typical threshold and image rendering settings. Adjustment of the threshold and rendering settings to high levels allow better definition of individual brain areas, as insular correlation to the ACC and posterior cingulate cortex (PCC) can be seen for the high-threshold case of cyclic pain. Statistical comparison of rest to cyclic pain shows that there were no areas where rest correlation exceeded that of cyclic pain; instead, it appears that cyclic pain results in increased correlation across the entire brain. With high thresholding, some individual areas of increased correlation can be identified, including the contralateral ipsilateral insula, the ACC, PCC, and cerebellum.

With GSR, several key differences can be readily observed in Figure 2, which is thresholded at $Z > 4$. First, the overall specificity of the correlation map is increased. Thresholding the non-GSR images of Figure 1 with Z-scores in the 10–25 range can create images approaching the specificity of the GSR images of Figure 2, but the identification of the individual Z-score peaks are still hampered by the conjoining of correlated areas when GSR is not used. This is not the case after GSR, where distinct areas are detected.

Second, as expected, GSR introduces negative correlations. The significantly correlated (and anticorrelated) areas are presented in Table 1. In the resting state, strong negative correlations to the insula can be found in the PCC, medial and dorsolateral prefrontal cortex, and cerebellum. During cyclic pain, negative correlations appear in a separate area of the PCC, prefrontal cortex, temporal lobes, and cerebel-

lum. Positive correlations to the insula are similar across rest and cyclic pain in S1, S2, and bilateral insula. One notable difference, however, is that cyclic pain does not possess the positive insula-to-ACC correlation found in rest.

Interestingly, when the with-GSR rest and pain maps are statistically compared, several key differences arise that were not appreciated in the non-GSR comparisons. The premotor, S1, ACC, S2, and bilateral insula cortices show positive correlations to the contralateral (left) insula seed region that are greater during rest. Due to the negative correlation to the insular seed seen for both the PCC and the cerebellum in rest, with no significant anticorrelation seen in the corresponding brain areas during pain, the pain > rest comparison maps show a significantly greater correlation between these areas during pain processing in the cyclic pain task.

To further examine this, the average coefficient of the parameter estimate (COPE), for correlation to the seed time course from the left insula (contralateral to the pain stimulus), was extracted from ROIs in the right (ipsilateral) insula, ACC, and PCC for both the rest and cyclic pain data. The relative values of the COPEs between the two conditions are shown in Figure 3. The results for the ipsilateral insula are easy to interpret in the context of the correlation maps since both COPEs (which correspond to the correlation coefficients) are positive; correlation was greater between the insula cortices during rest than during cyclic pain. Significant correlation to the ACC was not seen during cyclic pain, while ACC connectivity is seen on the rest map; thus, this area's appearance on the rest > pain map is expected and correlates to the COPE differences shown in the middle section of Figure 3. However, with GSR, the PCC gives an example of ambiguity in interpretation, as negative correlation to

FIG. 2. Selected slices (same as in Fig. 1), illustrating that GSR does introduce areas of negative correlation (anticorrelation), shown in blue. Notice that the ability to resolve individual brain areas is increased after GSR. The threshold for significance was $Z > 4$ for both positive and negative correlations. Color images available online at www.liebertpub.com/brain

the contralateral insula is seen during rest, with less negative correlation seen in cyclic pain. This results in an apparently greater connectivity during pain, as demonstrated by the pain > rest comparison map in Figure 2.

Effect of TPR

The next investigation was to determine if low-pass temporal filtering was sufficient to remove task-induced signal change. Basic preprocessing consisted of motion correction, spatial smoothing, and low-pass filtering as described in the methods. Examination of the seed (contralateral insula) region's signal after preprocessing shows that while low-pass filtering does smooth fluctuations in the signal and removes the sharp increases in the fMRI signal with stimulus initiation, the time course still has obvious task-induced activity. A typical example from one subject is shown visually in Figure 4. Even after low-pass filtering, stimulus-correlated MR signal increases can clearly be seen.

In the above analysis of the effect of GSR, the cyclic pain versus rest comparison was carried out using TPR. The cyclic pain task was also analyzed with and without TPR, and difference maps between cyclic pain and rest are shown in the first two columns of Figure 5. Of particular interest is that areas typically activated in task-based studies of pain, includ-

ing S1, S2, bilateral insula, and the ACC, all showed greater connectivity during pain processing than rest when TPR was not used. With the inclusion of TPR, the opposite relationship is seen, suggesting task-induced changes affect correlation analyses. Comparison of the cyclic pain maps versus rest difference maps to the corresponding tonic pain versus rest maps shows similarity between the paradigm-free tonic pain comparison and the paradigm-regressed cyclic pain + TPR comparison.

Discussion

In this study, we sought to investigate the effects of GSR and TPR on the analysis of insular functional connectivity changes between rest and acute experimental pain stimulation. Both GSR and TPR have been inconsistently applied to fMRI analysis, but no direct comparison has been performed. We show that both GSR and TPR had an effect on the resulting connectivity maps and are particularly impactful when comparing stimulation to rest, exemplified with experimental painful electrical nerve stimulation.

The use of GSR as a means of estimating and correcting for both physiologic and scanner (nonphysiologic) noise has been investigated since the late 1990s, with many of the investigations discussing the appearance of anticorrelations after GSR has been applied. GSR creates a bell-shaped distribution of correlation coefficients centered on zero, thus mathematically resulting in negative correlations with statistical analysis (Murphy et al., 2009) and a lower absolute value of correlation (Yeh et al., 2015). Obviously, knowledge of this transformation of the data must be used when interpreting GSR connectivity results. In addition, GSR resulted in improved specificity of detection of connectivity, as evidenced by smaller more discrete clusters of activation. However, investigators have shown that anticorrelations, especially between the DMN and the dorsal attention network, reflect biological processes as they are present in both component-based and GSR data (Chai et al., 2012) and these can exist before GSR (Fox et al., 2009). In this study, we have heeded the suggestion of Van Dijk et al. (2010) to not over-interpret negative correlations, as we are concerned less with the biological origins of the anticorrelations. Rather, the effect of these preprocessing steps on the interpretation of pain versus rest connectivity difference maps is the focus of this investigation. The importance of fully characterizing these anticorrelations with GSR is exemplified by examining the PCC. Looking at Figure 2, there is a negative correlation in the PCC at rest, but none in the PCC during pain. Without knowing that the PCC was an area with negative connectivity to the insula during rest (for example, if only the positive connectivity map was displayed), with no correlation during acute pain, the pain > rest difference map would be puzzling. The pain > rest difference is shown to arise from a less negative correlation during pain by the COPE comparison in Figure 3. The PCC's appearance as an area where correlation was greater during pain is due to the shift away from being strongly negatively correlated in the rest task.

When TPR was used, the difference maps comparing the cyclic pain and the tonic pain tasks to rest were comparable. Without TPR, the cyclic pain versus rest map suggests a very different interpretation, with opposite findings in many areas. This is likely due to task-induced BOLD signal changes,

TABLE 1. FUNCTIONAL CONNECTIVITY CLUSTER COORDINATES (IN THE MNI-152 STANDARD BRAIN) AND MAXIMUM Z-SCORES (Z-MAX) FOR ANALYSIS OF THE REST AND CYCLIC PAIN TASKS, USING THE LEFT INSULA SEED REGION

Task	Brain region	Laterality	Z-Max	Coordinates (mm)			BA
				X	Y	Z	
Rest (+)	Insula	L (contra-)	27.9	-42	-4	8	13
	Insula	R (ipsi-)	14.7	40	-4	4	13
	S2	L	13.5	-62	-12	20	40
	ACC (24)	R	9.64	2	8	34	24
	Premotor	Midline	9.50	0	0	52	6
	V3	L	8.33	-58	-72	8	19
	ACC (32)	L	6.23	-6	36	6	32
	Fusiform gyrus	R	5.26	56	-60	-18	37
	Midbrain	Midline	5.62	0	-16	-18	
Rest (-)	SAC	L	-11.6	-2	-66	40	7
	PCC (31)	Midline	-9.21	0	-48	36	31
	Cerebellum	L	-9.43	-14	-80	-40	II
	MPFC	R	-7.86	10	64	18	10
	Premotor	R	-10	26	26	54	6
	Premotor	L	-5.96	-12	14	54	6
	Orbital	L	-8.16	-48	34	-16	47
	Angular gyrus	R	-7.2	44	-62	36	39
	S. Temporal	R	-6.68	42	18	-38	38
Cyclic pain (+)	Insula	R	18.4	40	-34	-4	13
	Insula	L	15.8	-60	-24	8	13
	V1	R	9.74	6	-64	6	17
	Cerebellum	L	9.03	-38	-72	-20	I
Cyclic pain (-)	DLFC	L	-11.2	-26	62	18	10
	Premotor	R	-9.64	24	30	54	6
	DLFC	R	-9.58	36	62	16	10
	Cerebellum	L	-10.5	-10	-84	-52	II
	Cerebellum	R	-10.3	34	-82	-38	II
	PCC (23)	R	-9.61	2	-16	32	23
	PCC (31)	Midline	-8.02	0	-46	28	31
	M. Temporal	R	-12.6	64	-6	-26	21
	SAC	L	-7.77	-2	-68	50	7
	I. Temporal	L	-9.01	-62	-12	-24	20
	Orbital	R	-7.11	34	22	-20	47
	V2	R	-7.59	34	-92	4	18
	Orbital	L	-6.9	-12	12	-26	47

Results are further subdivided by whether the correlation was positive (+) or negative (-). Anatomical abbreviations not specified elsewhere: V3, visual cortex; SAC, somatosensory association cortex; DLFC, dorsolateral frontal cortex; V1, visual cortex; S. Temporal, superior temporal cortex; I. Temporal, inferior temporal cortex.

which are inadequately removed with low-pass temporal filtering alone as demonstrated. In Figure 5, the results show the striking difference in the comparison maps that occurs when TPR is used. Assuming that the tonic pain task represents functional connectivity during pain processing without the contribution of task-related signal changes, the similarity between tonic pain and cyclic pain with TPR demonstrates that TPR is needed for fMRI during task performance. That is, task versus rest connectivity difference maps can show drastically different results where task-induced changes continue to dominate the BOLD signal time course. This is likely to be the case in most fMRI studies during task performance, as connectivity changes are expected to overlap with areas that have a strong task-induced BOLD response. Our results support the use of both GSR and TPR when analyzing functional connectivity changes during task performance, as well as the need

to examine and present findings of negative connectivity for completeness when publishing results.

As mentioned in the introduction, determining functional connectivity during an experimental task is complicated by the superposition of the task-induced signal changes on the underlying low-frequency fluctuations interpreted as functional connectivity (Fox and Raichle, 2007). Several approaches to account for this have been used. Assuming that this superposition is linear (Fox et al., 2006), regression of the task signal out of the data could be expected to provide results that are easier to interpret. Arfanakis et al. (2000) showed that functional connectivity in areas not involved in the task was unaffected and suggested that areas activated by the task could be used for functional connectivity when the task-induced changes were removed with independent component analysis. Fair et al. (2007) tested this further by first using general linear modeling to remove the task-induced

FIG. 3. Coefficient of parameter estimate (COPE) values for functional connectivity of three areas (abbreviations defined in text) to the left insula. The average value across the anatomical region of interest is displayed graphically for rest (black bars) and pain (white bars). The relative COPE differences between the conditions and in reference to zero explain the pain versus rest difference maps (Fig. 2), as discussed in the text.

signal from an event-related paradigm and then analyzing the functional connectivity of the residuals. They found that the residual method showed qualitatively similar results, but differed from continuous data in several significant regions, leading us to directly regress the signal as part of the same model in this study.

In terms of brain functional connectivity, our results suggest that the insular cortex contralateral to an experimental electrical pain stimulus exhibits decreased connectivity to the ACC and increased connectivity to the PCC. Based on the PCC's prominence in the default mode network (DMN), our results also suggest a possible increase in insular to DMN connectivity during acute pain. Only a few other studies have used fMRI to study acute pain processing in healthy volunteers. Kim and colleagues (2013) used a tonic painful pressure task to assess connectivity changes during pain, although they did not find connectivity differences with relation to the DMN between rest and pain. One possible explanation for the apparent difference in the DMN findings between their work and ours is our use of a seed-based analysis, in contrast to an ICA-based

FIG. 4. The effect of low-pass filtering for a representative subject. The stimulation paradigm timing is shown by the thick black lines at the bottom of the graph. The raw signal time course for the left insula (before filtering) is shown in gray. After preprocessing that includes low-pass filtering, strong stimulus-correlated signal increases still remain as shown in black, demonstrating that temporal filtering alone does not remove task-induced signal changes.

FIG. 5. Overlaid connectivity difference maps, comparing cyclic pain with (+) and without (-) TPR and tonic pain to rest. Color bars represent Z-score for the significant differences of pain > rest (red-yellow) and rest > pain (blue-green). Color images available online at www.liebertpub.com/brain

approach for network identification. Due to their model-free design, ICA approaches are able to identify significant signal fluctuations that occur over the course of a scanning session in an exploratory manner (Beckmann et al., 2005) at the expense of specificity in the hypothesis definition. Since we

were focused on preprocessing effects on functional connectivity, we needed to ensure a consistent approach between analysis techniques and across three data sets, and thus, a seed-based technique was required.

The key finding of Kim and colleagues (2013) is that their experimental painful stimulus altered the functional connectivity between the salience network (which includes the ACC and the insula) and S1. Furthermore, there was a specific suggestion that the right anterior insula is the key to these changes when analyzing their data with an S1-seed-based algorithm. Our data support this parcellation of processing within distinct regions of the insula. In another study, using a cyclic heat pain task, Peltz and colleagues (2011) showed that the insula is divided into anterior and posterior regions that possess different functional connectivity characteristics. Our insula seed region occupies a more posterior position relative to that of Kim and colleagues (2013); since Peltz and colleagues (2011) describe differential somatosensory connectivity when comparing the posterior and anterior insula, our results seem compatible. Future investigations of insular connectivity will likely require precise specification in regard to anterior/posterior positioning.

One limitation of our study is the relatively short scanning times of 3.5 min for the resting-state data and 4.5 min for the cyclic and tonic pain data, since 6 min for resting-state connectivity analyses has been postulated as being optimum (Van Dijk et al., 2010). The 30-sec on/off cycle was chosen because it has been commonly used with ENS, is well characterized, and was felt to be a logical candidate as a cyclic stimulus to evaluate the effects of TPR. The 2-min tonic stimulation was used to keep the overall length of the painful stimulation constant for the cyclic versus tonic comparison. While the effect of longer scanning time is not certain, the agreement between our data and that of Kim and colleagues (2013), who used a 6-min stimulus, suggests that the differences would be small. Furthermore, the effect of the short scanning time is most likely an increase in noise within the data and a subsequent masking of small differences between the rest and task images. Thus, if this experiment was repeated with longer scanning times, the differences found within would likely hold, and other areas would be identified.

Another limitation worth noting for TPR is that our chosen task was only investigated for a 30-sec ON-OFF paradigm, with a total cycle period of 60 sec. However, there could be an interaction between the task period length and the relative effects that TPR and low-pass filtering would have on the data. Certainly, at faster cycling frequencies or shorter task periods, the low-pass filter can be set more aggressively, possibly changing the relative noise reduction from GSR and TPR. However, this does not necessarily imply that the need for TPR is obviated, as irregular and infrequent task-induced BOLD signal changes may still want to be considered independent of functional connectivity during task performance. As more investigators move toward event-related (and other more complex) experimental paradigms, it would be interesting to repeat our comparative analysis of the data processing pipeline for such data sets.

A final element of our implementation of TPR worth further discussion is our use of a gamma hemodynamic response function convolved with a boxcar design for the regressor, rather than the optimized multipeak model discussed in a prior pain fMRI publication (Ibison and Vogt, 2013). The specific timings and relative amplitudes of the multiple

peaks in that model varied across the brain (and likely would vary for different pain tasks), just as the BOLD hemodynamic response varies across the brain. Therefore, to maintain generalizability, we chose the standard gamma function convolved boxcar model and found good agreement between the with TPR cyclic pain and the tonic pain tasks, as hypothesized. While the sensitivity of TPR to small variations in the shape of the regressor should be investigated, we suspect that more specific models will not prove to be superior across the entire brain. However, if a study's goal was to focus on a specific area, then a multipeak model for the BOLD response to pain should be considered.

Finally, an important limitation of the current study is its scope: one seed region (the left posterior insula) was analyzed at rest and during two durations of painful ENS. This area of the brain is clearly important in processing pain and other somatosensory stimuli (Peltz et al., 2011). The diffuse connectivity for the posterior insula that we have shown and discussed suggests that our work may be generalizable to investigations of other brain areas. However, an exhaustive evaluation of multiple brain areas for multiple tasks was beyond the scope of this work. Our goal was to demonstrate the impact of several possible optimizations of an fMRI analysis pipeline for an experimental pain data set. Using our methods as a guide, investigators can determine the extent to which our results apply both to their own work and results found in the literature.

Conclusions

We have demonstrated several key findings related to seed-based fMRI analysis using the insular cortex contralateral to the stimulation for BOLD fMRI data acquired during rest and experimental pain stimulation. The use of GSR reduces the size and statistical significance of connectivity clusters. Furthermore, GSR adjustment of the baseline MR signal creates negative correlation coefficients for some connectivity clusters. Exploring the presence and relative relationships of the negative correlations is particularly important when comparing connectivity between two data sets. We also demonstrated that regression of the task paradigm of a cyclic stimulation gave connectivity results similar to those with a constant task, suggesting that analysis, including TPR, is more accurately reflective of neuronal activity. Finally, pain versus rest connectivity from the examined (posterior) portion of the insula contralateral to the stimulation showed increased connectivity to the PCC during pain and decreased connectivity to the ACC, S1, S2, and ipsilateral insula, consistent with prior studies. This work, in a modest number of healthy subjects, examined one brain area as the seed region for functional connectivity during pain as an experimental task. However, it provides a framework within which future investigators can critically analyze the effects of GSR and TPR on their own work and aid in interpreting the published works of others.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

James W. Ibinson
Department of Anesthesiology
Center for Pain Research
University of Pittsburgh
5750 Centre Avenue, Suite 400
Pittsburgh, PA 15206

E-mail: Ibinsonjw@upmc.edu