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Functional Magnetic Resonance Imaging Studies of Pain

An Investigation of Signal Decay during and across Sessions

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Background: **Several investigations into brain activation caused by pain have suggested that the multiple painful stimulations used in typical block designs may cause attenuation over time of the signal within activated areas. The effect this may have on pain investigations using multiple tasks has not been investigated. The signal decay across a task of four repeating pain stimulations and between two serial pain tasks separated by a 4-min interval was examined to determine whether signal attenuation may significantly confound pain investigations.**

Methods: **The characteristics of the brain activation of six subjects were determined using whole brain blood oxygenation level–dependent functional magnetic resonance imaging on a 1.5-T scanner. Tasks included both tingling and pain induced by transcutaneous electrical stimulation of the median nerve. The average group maps were analyzed by general linear modeling with corrected cluster *P* values of less than 0.05. The time courses of individual voxels were further investigated by analysis of variance with *P* values of less than 0.05.**

Results: **Significant differences between pain and tingling were found in the ipsilateral cerebellum, contralateral thalamus, secondary somatosensory cortex, primary somatosensory cortex, and anterior cingulate cortex. Highly significant signal decay was found to exist across each single pain task, but the signal was found to be restored after a 4-min rest period.**

Conclusions: **This work shows that serial pain tasks can be used for functional magnetic resonance imaging studies using electrical nerve stimulation as a stimulus, as long as sufficient time is allowed between the two tasks.**

INTEREST in functional magnetic resonance imaging (fMRI) to map brain activation is rapidly growing because of the tremendous potential of this technique for neuroscience research and the wide availability of magnetic resonance (MR) scanners. fMRI is especially intriguing in anesthesia research, where fMRI shows the potential to illustrate the brain areas affected by anesthetic agents.[1] Briefly, the blood oxygen level–dependent (BOLD) fMRI effect is due to changing concentrations of deoxyhemoglobin associated with brain activation. Neuronal activity causes a localized increase in metabolism and thus an increase in the demand for oxygen. The local blood vessels respond to this need with a disproportionate increase in regional cerebral blood flow. The increase in blood flow overcompensates for the increase in oxygen demand and causes a decrease in the concentration of deoxyhemoglobin.[2] Deoxyhemoglobin is paramagnetic and induces local magnetic field inhomogeneities that decrease the detected MR signal. Thus, neuronal activation causes deoxyhemoglobin concentration decrease and local signal increase. These localized MR signal increases can be used to identify activated areas of the brain by comparing images collected at rest to those collected while the subject performs a task.

Of great importance for future successful fMRI studies of pain is characterization of the impact of painful stimuli on a BOLD fMRI experiment. Several studies have used fMRI to investigate the effect anesthesia has on pain-induced brain activation by gathering images during a baseline pain task, administering an anesthetic agent, and then repeating the pain task.[3,4] However, caution is necessary in interpreting these results because painful stimuli have been shown to cause BOLD fMRI signal attenuations that could confound the results.[5,6] These studies showed that pain-induced BOLD signal changes decrease in amplitude each time the painful stimulus is repeated. If the attenuation of the pain-induced BOLD signal from the baseline task in the example above persisted into the second pain task, it would be impossible to determine whether decreased brain activity in the second task was due to this attenuation or due to the intervention, thereby confounding the results of this (and all) investigations using serial pain tasks. The major objective of this work was to investigate whether two serial pain tasks, separated by a reasonable amount of time, would show a significant difference in their activation maps due only to signal change attenuation. To accomplish this, it would first have to be shown that the BOLD signal for electrical nerve stimulation (ENS)–induced pain contained the attenuation pattern demonstrated in other studies.[5,6]

For this study, ENS was used to induce pain.[7] ENS-induced nonnoxious stimulation has a vibratory (tingling) characteristic and can provide an appropriate con-

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trol for pain imaging. Vibratory sensations have been shown to activate the contralateral primary somatosensory cortex (SI), the secondary somatosensory cortex (SII) bilaterally, and the insula.[8] fMRI of pain has consistently shown activation in SII, the insula, the anterior cingulate cortex (ACC), and the cerebellum and often but inconsistently in SI and the thalamus.[9,10] Considering the spatial distribution of these areas, this work used a whole brain BOLD fMRI technique to determine the differences in the activation patterns of both a nonnoxious ENS task and a noxious ENS pain task. However, using a whole brain technique raises an important question. Early theoretical considerations[11,12] showed that the sensitivity to the BOLD effect is a function of the "time to echo" (TE, an imaging parameter that affects the signal intensity) and that the TE must be equal to the imaged tissue's T2* relaxation time to maximize BOLD signal.[11] It has been shown that the T2* of the human brain can vary considerably across its volume, ranging from 40 to 80 ms.[13] For a whole brain experiment, this implies that it is not possible to maximize BOLD contrast throughout the brain using a pulse sequence with a single TE. Other nonpain studies have shown the BOLD signal to vary considerably as a function of TE.[14,15] Therefore, the final objective of this study was to determine whether the choice of TE affected the BOLD fMRI activation map for pain for scans at 1.5 T.

In summary, three specific questions have been investigated: (1) to verify that BOLD signal change attenuation occurs over the course of a 4-min study, (2) to determine whether this attenuation is still present after a 4-min rest period, and (3) to test whether the choice of TE has the ability to affect the activation pattern seen for the protocol used herein.

Materials and Methods

Six normal subjects (three men and three women, aged 25–48 yr, mean age of 31 yr) were recruited in a study approved by The Ohio State University Institutional Review Board (Columbus, Ohio). Written informed consent was obtained from all subjects. All were right handed and free from neurologic disease. All subjects had previous experience with fMRI studies and denied recent use of analgesic or vasoactive substances.

Stimulation Protocol

Electrodes were placed on the right wrist and forearm to stimulate the median nerve. The median nerve is responsible for carrying sensation from the thumb, the complete first and second fingers, and the lateral half of the third finger to the spinal cord. A standard intraoperative nerve stimulator (Ministim Model MS-III; Life Tech, Stafford, TX) was connected to the electrodes. The nerve stimulator outputs a biphasic wave at 100 Hz.

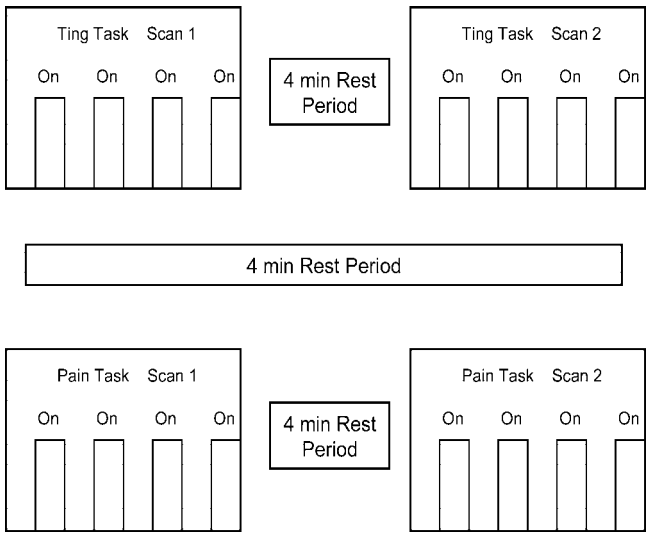

Fig. 1. Illustration of the study design. Two tasks (tingling and painful stimulation) were imaged during each session. Each task consisted of four 30-s stimulation periods alternating with four 30-s rest periods. Each task was repeated twice. Four minutes evolved between each task. All four functional scans were then repeated in two additional sessions, for a total of 12 functional scans.

Stimulation intensity was rated using a verbal scale.[16] ENS was first administered at low current to establish that the median nerve was being activated. All subjects described the perception of this low-intensity stimulation as a strong tingling sensation. The intensity was then increased until the subject reported a verbal scale pain rating of 5. The setting on the nerve stimulator was noted, and the intensity was reduced to a level again described as a strong tingling sensation with a verbal scale pain rating of 0.

Two tasks were used for this investigation: an ENS-induced tingling task and an ENS-induced pain task. The tingling task served as the control against which painful stimulation activation could be compared. It was always imaged first. After a 4-min rest period, functional images of pain were collected by increasing the intensity of the nerve stimulator to the predetermined verbal scale level of 5. Each task was performed twice before moving on to the next. Four minutes always evolved between repetitions, and the subjects were asked to remain motionless during this time. Therefore, a scanning session consisted of four sets of functional data: two tingling sets followed by two pain sets.

Each task consisted of four epochs of 30 s of rest and 30 s of task (electrical stimulation). The TE used for each functional scan was randomly assigned to be either 40 or 60 ms because these are the range of values commonly used. Finally, each subject was asked to return for two additional complete sessions, each on different days, giving a total of 12 functional data collections for each subject (and 72 total sets). The design is summarized in figure 1.

Functional Imaging Protocol

Each time the subject entered the scanner, a localizer scan was collected to establish the subject's position within the 1.5-T General Electric (Milwaukee, WI) Signa scanner. A high-resolution steady state T1-weighted three-dimensional scan (repetition time, 20 s; flip angle, 40; matrix, 256 × 128; field of view, 24 cm; slice thickness, 2.5 mm; 60 axial slices) for use as an anatomical underlay for the functional data that was then collected. The functional scans followed and were collected with a gradient echo, echo-planar imaging technique (repetition time, 3,000 ms; TE, 40 or 60 ms; flip angle, 90; matrix, 64 × 64; field of view, 24 cm; in-plane resolution, 3.75 × 3.75 mm; slice thickness, 5 mm; 28 axial slices; 83 images of each slice) with EPIBOLD software provided by General Electric Medical. Using these parameters, the entire brain was scanned. This gave a total functional scan time of 4 min 9 s and a total session time of approximately 1 h. The first three volumes of each scan were discarded because these were collected during the establishment of a steady state MR signal.

fMRI Data Preprocessing

Data were reconstructed off-line using EPIRECON software also provided by General Electric Medical. During reconstruction, a Fermi filter with a width of 10 mm and radius of 32 mm was used to enhance signal-to-noise ratio. The functional images were analyzed using the FMRIB Software Library (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, John Radcliffe Hospital, Headington, Oxford, United Kingdom). Preprocessing consisted of motion correction,[17] nonbrain signal removal,[18] and spatial smoothing using a gaussian kernel with a full-width half maximum of 5 mm. In addition, grand mean-based intensity normalization was performed. This scales the entire data set by a single scale factor to insure validity of group analyses across subjects. Furthermore, high-pass temporal filtering (gaussian-weighted least squares straight line fit with sigma = 45.0 s) is performed to remove drift in the baseline signal.[19] Finally, the images are registered to a standard brain.[17]

As mentioned above, all 72 data sets were processed to quantify and correct for subject motion. Data were excluded if it was calculated that the subject's head moved more than 1 mm from one image to another. In addition, correlation coefficients were calculated between the motion estimates and the stimulus paradigm. Data were excluded if the correlation coefficient was greater than 0.22 (the value necessary to give a voxel-wise P value of 0.05), indicating significant task-correlated motion (figs. 2A and B). Furthermore, after each task, subjects were asked whether they were able to maintain mental focus on the task. If not, the data were excluded from further investigation, because attention has been shown to affect brain activation.[20]

In all, 22 (11 from the tingling task and 11 from the pain task) of the 72 collected data sets were excluded from further analysis, leaving a total of 50 sets. The excluded sets were well distributed over all six subjects (subject 1: two sets total for tingling and pain; subject 2: two sets; subject 3: six sets; subject 4: five sets; subject 5: three sets; subject 6: four sets). The distribution indicated a trend toward exclusion of sets collected at the end of the study, suggesting that fatigue played a role in subject motion with the stimuli and possibly in their ability to maintain mental focus on the task. Of the 22 excluded, 8 were for stimulus-correlated motion, 8 were for random motion of over 1 mm in magnitude, and 6 were for failure to maintain mental focus on the stimulus.

Statistical Analysis: fMRI Group Activation Maps:

For individual subject analysis, the data from each functional scan were independently analyzed using General Linear Modeling with the FMRI Expert Analysis Tool[21] Version 5.1 (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain) by generating task-induced z-score maps. These z-score images were then used to calculate average activation maps for both tingling and pain using clusters with $z > 2.0$ and a corrected significance of $P = 0.05$. The individual subject maps were also analyzed as a group with the FMRI Expert Analysis Tool using an ordinary least squares simple mixed effects analysis of variance between groups (ANOVA) model. Areas of significant brain activation for the ANOVA model were determined using clusters identified by a $z > 2.3$ threshold and a corrected cluster threshold of $P = 0.05$.[22–24]

The ANOVA model examined TASK (two levels: tingling or pain), TE (two levels: 40 or 60 ms), and SCAN (two levels: first scan or second scan of each task for each day). All main effects and interaction terms were included. The TASK main effect highlights brain areas that are significantly different between the tingling and the pain tasks. Presence of a significant TE main effect would suggest that the choice of TE affects the activation map. The SCAN main effect assessed the overall repeatability of the activation maps. Signal change attenuation due to pain persisting over the 4-min rest period between sessions would be evident by a significant SCAN main effect or interaction term. Areas exhibiting a significant effect in the ANOVA were further investigated by inspection of the group average activation maps to determine their nature.

Statistical Analysis: MR Signal Time Courses:

Individual MR signal time courses were also examined for attenuation. To accomplish this, each subject's preprocessed data sets described above were also analyzed with the Analysis of Functional NeuroImages[25] software (Robert W. Cox, National Institute of Mental Health,

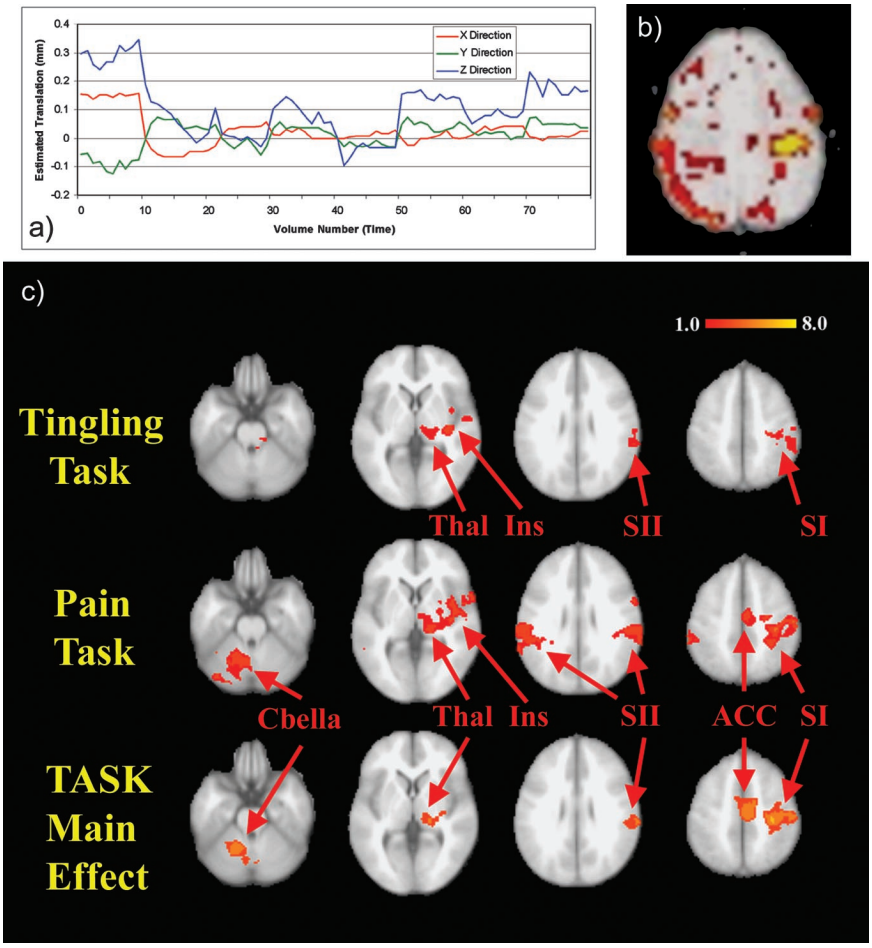

Fig. 2. (*A*) Time course of calculated voxel displacements illustrating an example of stimulus correlated motion. Pain was induced for volumes 10–20, 30–40, 50–60, and 70–80. Substantial movement occurred each time the pain stimulus occurred. (*B*) This type of motion results in the artifactual ring of activation as shown. Data that displayed this type of motion, as determined by correlation analysis between the movement estimates and the stimulation timings, were excluded. (*C*) Illustration of the key areas found to be activated by the tingling and the pain tasks and those areas that showed a significant difference between the two (*i.e.*, areas that possessed a significant TASK main effect). The other factors of the analysis of variance model (time to echo, SCAN, and all interaction terms) were not significant and are not presented here. Shown are the anterior cingulate cortex (ACC), thalamus (Thal), insula (Ins), cerebellum (Cb), primary somatosensory cortex (SI), and secondary somatosensory cortex (SII). The peak z scores for each area are given in table 1, along with their coordinates. Coloring of activated areas represents z scores using the scale shown. Images are shown with anatomical right on the image's left.

Bethesda, MD). Activated voxels were determined by correlation analysis with a boxcar ideal waveform shifted by 3 s to account for the hemodynamic delay present in BOLD fMRI. The most strongly correlated single voxel was identified in the ACC, SI, and the cerebellum. For each area, the signal time courses from these voxels were then averaged with respect to scan, *i.e.*, subjects' first pain scans of each day were averaged together, as were the signal time courses for each subject's second scan. The average signal change for each task period was calculated using the last 9 of the 10 points gathered during each task period, because the first point was collected during the development of the BOLD signal. This average was input into Statview (SAS Institute Inc., Cary, NC) as a two-factor ANOVA to allow investigation of both the immediate signal change attenuation demonstrated by Becerra *et al.*[5] and Kurata *et al.*[6] and the across-scan attenuation with which this investigation is concerned. These factors were named STIMULATION (with four levels, one for each of the painful electrical nerve stimulations of the pain task) and SCAN (with two levels: first or second). This is illustrated in figure 3 for one subject. Overall significance for each factor was set at $P = 0.05$, and a *post hoc* Fisher protected least significant difference test of all pairwise comparisons was used to test any significant factor. The analysis of the TE = 40 or 60 ms data was performed in a similar manner, except that averaging was done with respect to TE instead of scan.

Results

fMRI Group Activation Map Analysis:

The average group brain activation over all subjects due to the tingling task is shown in figure 2C and detailed in table 1. From this map, it can be seen that the only areas consistently activated are the contralateral SI and SII cortices, thalamus, and insula. This is in contrast to the group activation observed during painful stimulation, which shows activation in the ipsilateral cerebellum, the contralateral insular cortex, the thalamus, the ACC, and SI, and in SII bilaterally (fig. 2C and table 1).

Analysis of variance showed a statistically significant result due only to the TASK main effect (fig. 2C and table 1) in the ipsilateral cerebellum and in the contralateral thalamus, the insula, SII, SI, and the ACC. The presence of this effect indicates that pain and tingling exhibit distinctly different activation patterns, suggesting that pain and tingling sensations caused by electrical nerve stimulation are interpreted differently by the brain. This

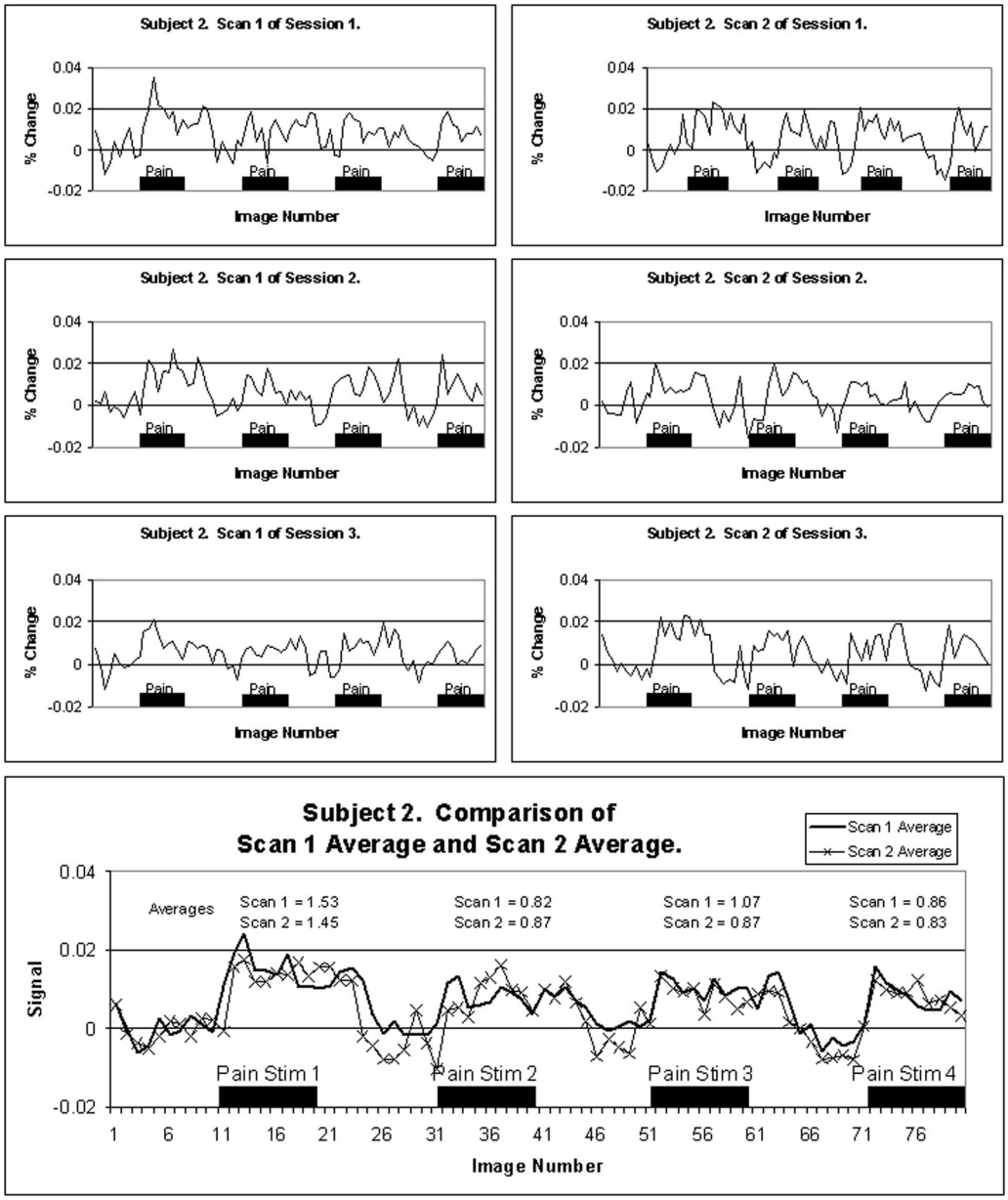

Fig. 3. Example of the time course analysis technique for one subject. Each of the six signal time courses for this subject's pain task are plotted, along with the calculated average time courses for the first scans of each session (scan 1) and the second scan of each session (scan 2). The signal change analyzed was the average of the last nine points for each of the painful stimulations. The average values for this subject are shown above each peak on the average time course plot.

difference can be either in activation intensity (z score) or in location. Examples of both are present in the data. The SI contains higher activation during pain than during tingling. Because both the pain and the tingling sensations were reported by the subjects to cover the same area of skin, this suggests that intensity encoding may be occurring in the SI. The ACC is an example of an area that shows activation only when pain is induced; it does not contain activation during tingling but does during pain. The TE main effect, the SCAN main effect, and all interaction terms were not shown to be significant; therefore, these maps are not shown.

Individual Voxel MR Signal Time Course Analysis:

The pain data were investigated for areas that resembled the signal attenuation pattern demonstrated by Becerra *et al.*[5] They found voxels with significant attenuation to their painful stimulus in the ACC, the insula, and the frontal gyrus but not in SI or the thalamus. This work studies the ACC (as it represents activation due to the affective/motivational aspect of pain), SI (to represent the sensory/discrimination aspect), and the cerebellum. ANOVA was performed using individual voxel time course data for both signal change attenuation within a scan and attenuation across the two separate scanning

Table 1. Maximum *z* Scores for Activated Areas

	Tingling Task				Pain Task				Task Main Effect			
	Coordinates			Max *z*	Coordinates			Max *z*	Coordinates			Max *z*
Brain Region	x	y	z	Score	x	y	z	Score	x	y	z	Score
Cerebellum, right	—	—	—	—	38	−80	−32	3.53	12	−56	−28	4.97
Thalamus, left	−20	−32	−8	4.05	−24	−22	8	3.03	−18	−24	0	4.67
Insula, left	−30	−2	−12	3.18	−46	−10	−6	3.59	—	—	—	—
SII, right	—	—	—	—	52	−24	12	3.01	—	—	—	—
SII, left	−48	−28	14	3.11	−64	−30	8	3.81	−68	−28	26	3.94
ACC, left	—	—	—	—	−8	−10	46	3.04	−14	−4	38	4.65
Premotor area	—	—	—	—	—	—	—	—	−6	−20	50	5.05
SI, left	−68	−24	36	3.09	−38	−30	50	4.63	−34	−28	52	5.64

The activate areas were taken from figure 4. The coordinates are given in millimeters for the MNI standard brain.
ACC = anterior cingulate cortex; SI = primary somatosensory cortex; SII = secondary somatosensory cortex.

sessions occurring 4 min apart. The average of all subjects' ACC time courses for the pain task is displayed in figure 4A. This graph demonstrates the signal change attenuation present. A significant difference was found for STIMULATION (which compared each of the four separate stimulations of the pain task; $P = 0.0208$) but not for SCAN (comparing the first scan of each session to the second; $P = 0.0864$). *Post hoc* analysis showed that the significant STIMULATION effect is due to differences between the first and the second stimulations ($P = 0.0382$) and between the first and fourth stimulations ($P = 0.0026$) for the ACC. Therefore, the BOLD signal change caused by pain decreases as the painful stimulation repeats, but a 4-min rest between scans seems long enough restore the signal to near original levels.

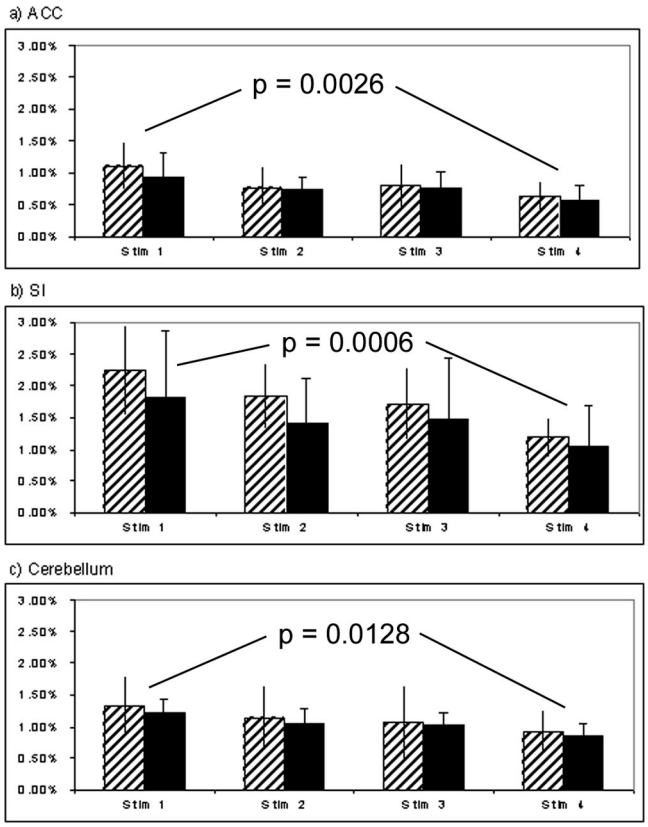

Fig. 4. Overall average magnetic resonance signal change due to the pain task for the (*A*) anterior cingulate cortex (ACC), (*B*) primary somatosensory cortex (SI), and (*C*) cerebellum for all subjects. *Cross-batched bars* represent data from the first scan, and *solid black bars* represent data from the second scan. The value for each stimulation was the average of the last nine points. *Error bars* indicate ±1 SD. The *P* values for all comparisons between stimulation 1 and stimulation 4 are shown in the figure; other significant comparisons are detailed in the main text.

The results for SI, as illustrated in figure 4B, are remarkably similar to those for the ACC. Again, there was not a significant effect due to SCAN ($P = 0.1218$), which suggests that attenuation does not persist across a 4-min rest. STIMULATION, however, was significant ($P = 0.0066$), and *post hoc* analysis reveled that the 1% signal change caused by the fourth painful stimulation was significantly lower than the 2% signal change of the first ($P = 0.0006$). These results contradict those of Becerra *et al.*[5], who found no significant SI signal attenuation.

The cerebellum data shown in figure 4C also suggest that there is not a significant effect due to SCAN ($P = 0.5469$). However, these data do not possess a significant overall STIMULATION main effect ($P = 0.0916$), even though there is a strong graphical suggestion of a trend in figure 4C. With this in mind, the *post hoc* Fisher protected least significant difference test of all pairwise comparisons was performed. This test again points to a strong attenuation in pain data, with $P = 0.0128$ for stimulation 1 *versus* stimulation 4 in the cerebellum.

As stated previously, the TE main effect was not shown to be significant in the global activation maps. However, by looking at the individual time courses of the cerebellum, SI, and the ACC with respect to TE (40 or 60 ms) in the same manner as described for attenuation above, a significant trend is observed (fig. 5). Neither data set contained activated areas that were not present in the other, explaining the lack of a significant effect in the global maps, but the data of figure 5 suggests that a TE of 60 ms results in greater signal changes.

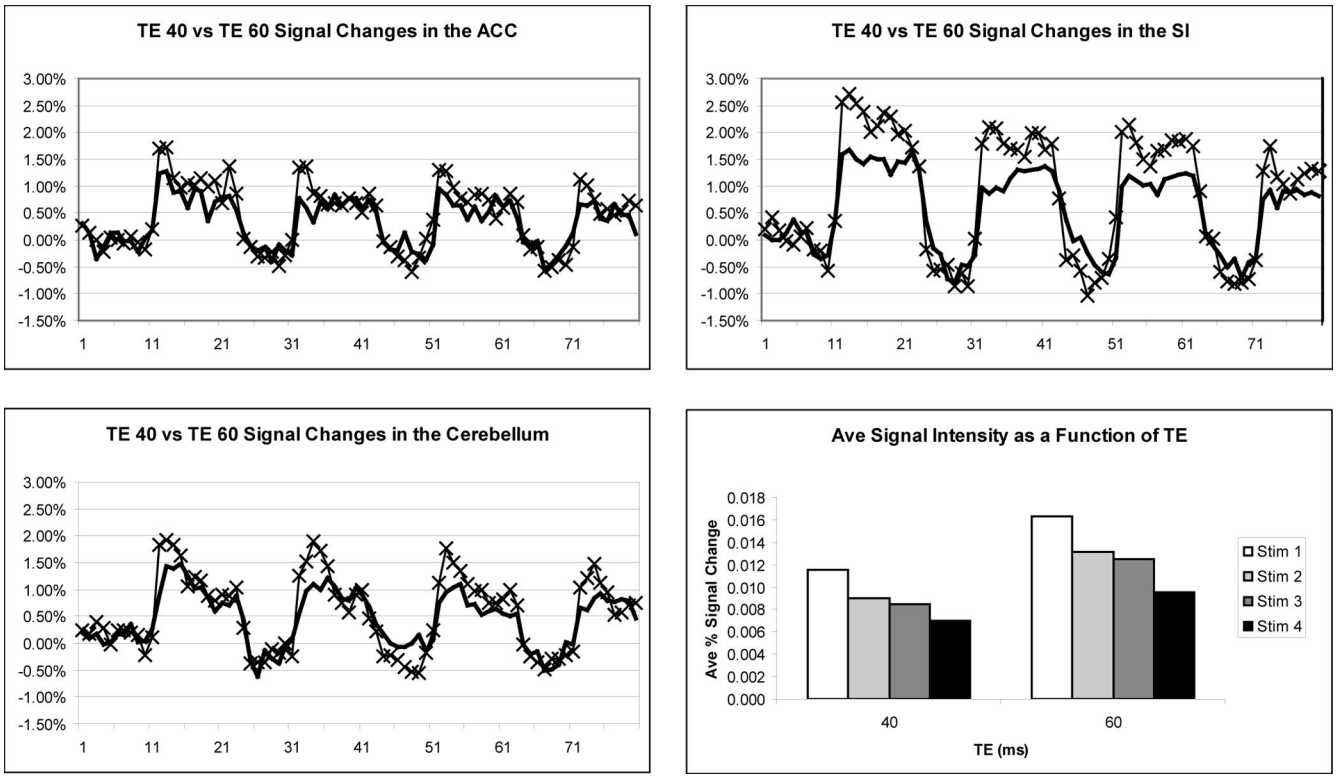

Fig. 5. Illustration of significant time to echo (TE) effect in individual voxel time course data for the cerebellum, primary somatosensory cortex (SI), and anterior cingulate cortex (ACC). TE = 40 data are plotted as *solid black lines*, and TE = 60 data are plotted as *lines with "x" markers*. The average signal intensity plotted in the bar chart is the average of all three areas and is given as percent change from baseline. The bar plot shows that TE = 60 provides a higher signal change when averaged over these three areas ($P = 0.02$).

Discussion

The perception of pain is complicated and involves numerous areas through both the peripheral and central nervous systems. The spinothalamic, spinoreticular, spinomesencephalic, cervicothalamic, and spinohypothalamic tracts carry painful sensations to the amygdala, the periaqueductal gray, and the thalamus, which then connects to the insular cortex, the primary somatosensory cortex, the basal ganglia, the motor cortex, and the posterior parietal cortex.[26,27] When nociceptive stimulations reach the thalamus and limbic levels, the percept known as pain begins to arise. This percept has three components: affective–motivational, sensory–discriminative, and cognitive–evaluative.[28]

Figure 2C shows that activation differed between the pain and tingling tasks in the ACC, the cerebellum, the thalamus, SII, and SI. The presence of areas where TASK would be significant was expected. The literature has repeatedly shown that the ACC is specifically involved in pain processing and is not normally activated by nonnoxious control stimulations.[29,30] The presence of the ACC (and the cerebellum) in the task comparison map is clearly a result of this fact. On the other hand, both tingling[31] and pain[9] have been shown to activate the thalamus, SII, and SI, and it was expected that the activation in these areas would cancel on comparison. The chief difference in these areas seems to be in activation intensity, suggesting it is dependent on stimulation strength. Coghill *et al.*[32] investigated stimulation intensity coding within the brain in depth, showing that the pain-induced signal change within activated areas of the brain was correlated to the intensity of the pain experienced. Among the investigated areas common to both studies, Coghill *et al.*[32] found this effect in the cerebellum, the thalamus, the insula, the ACC, SII, and SI. Therefore, stimulation intensity seems to be the factor causing the activation in the thalamus, SII, and SI to be significantly different.

Time to echo did not significantly affect the global activation pattern, because there were no areas of activation that were only present in the TE = 40 or 60 ms maps. This does not mean that an optimum TE does not exist for different areas of the brain. It simply means that the difference in BOLD sensitivity is not large enough to change the activation pattern at 1.5 T. However, there is a clearly increased BOLD signal change using 60 ms *versus* 40 ms, as shown in figure 5. This supports the findings of Gorno-Tempini *et al.*[15] in that longer TE values can provide better BOLD contrast even in the face of susceptibility effects and varying T2* values across the brain. Considering the small pain-induced signal changes shown in this article, any actions that can maximize signal should be taken.

As mentioned in the background, BOLD fMRI has been used to determine how various clinical interventions can affect brain activation caused by pain. Typically, a pain fMRI study is performed, the subject is given an intervention, and the fMRI study is repeated. The activation maps from the two pain tasks are then compared, and any changes are attributed to the treatment (or other tested intervention). If the BOLD signal change attenuation demonstrated here for each individual pain study persisted across the resting time between serial pain tasks, it would be impossible to tell whether activation was decreased due to habituation or due to the intervention. The results of this study clearly indicate that if a rest period of at least 4 min is observed between pain tasks, the attenuation can be more likely attributed to the intervention, not to some form of habituation.

If pain-induced BOLD signal attenuation was still present after the 4-min rest, a significant SCAN effect would have been seen. The lack of a significant SCAN effect shown here implies scan-to-scan attenuation is not present in normal, healthy individuals. SCAN was not found to be significant by either global activation map analysis or by analysis of individual signal time courses. However, this raises the possibility that this study did not have enough power to detect a difference in SCAN. Using the time course data, this issue was addressed. Power can be calculated when the number of subjects, the desired error rates, and the SD of the sample are known. The SD of the pain task stimulation periods expressed as a percent signal change was 0.3%. The signal difference between the first and the fourth stimulations was approximately 0.5%, and this is the difference this study desired to detect. Therefore, with six subjects, the power for testing SCAN is 0.72. This is acceptable and leads to the conclusion that there was enough power to see a difference within SCAN had there been one. Therefore, a 4-min rest between pain studies is enough to dissipate any signal attenuation. This finding validates the use of serial pain tasks in BOLD fMRI studies.

Finally, the decrease in pain-induced signal change within a single scan supports the findings of Becerra *et al.*[5] and Kurata *et al.*[6] In their work on the time course of the BOLD fMRI signal of pain, Becerra *et al.*[5] noted that the BOLD signal change was affected by a previous pain task. Using a block design where 30-s pain presentations were separated by 30-s rest periods, they found that by the third presentation, the pain-induced signal change had attenuated to the point where almost no change could be observed. The correlation coefficients of the MR signal in each activated area to a model that predicted equal BOLD signal changes from all four stimulations was significantly reduced relative to a model that used only the first two stimuli. Becerra *et al.* reasoned that the signal change attenuation was the result of changes in neural activity and postulated that either descending analgesia systems or temporal and spatial dispersion in afferent C-fiber input may be the source. In similar work, Kurata *et al.*[6] also found that the time course for pain data showed signal attenuation when compared to finger tapping and visual saccade tasks. They termed this *early decay* and suggested that it was the result of either a pain-induced global cerebral blood flow decrease or activation of the descending analgesia systems.

The data of this study show that signal attenuation was significant for SI and the ACC, and a trend was suggested for the cerebellum. This was especially clear when comparing the first to the fourth stimulation. This has great implications for the analysis of pain data. Statistical analysis that assumes a constant signal change with stimulation will be severely affected when the stimulus induces decay. The signal change in many of the subcortical areas involved in pain processing is low (on the order of 1% signal change). When this is combined with errors in analysis due to neglecting the signal decay, significant artifact may be introduced. Therefore, future analyses of fMRI pain data may benefit from using a decaying model consistent with the signal decay demonstrated.

The data from this study was reanalyzed using the decaying model described above with the technique described in the methods for group activation mapping. The activation pattern did not significantly differ from the pattern shown in figure 2C. However, the peak z scores found by the decaying model ($z = 4.97$) were higher than the peaks for the standard boxcar ($z = 4.63$), suggesting that the decaying model fits the data better. Using a decaying model did not provide a statistically different activation map, but that does not mean that the decay during each session is unimportant. It highlights an important difference between fMRI studies of pain and fMRI studies of functions such as vision: the BOLD response for pain studies may depend on the frequency and duration of the stimulus, whereas the BOLD response is often considered constant for tasks such as vision. The authors want to stress that the decaying model was developed from data using a specific stimulation protocol. The results may differ if a different method of pain induction (*i.e.*, heat) or a different pattern (something other than a 30 s on–30 s off pattern) is used.

Several causes for the signal change attenuation have been offered, including neural activity modulation by descending pain inhibitory mechanisms, nociceptor adaptation, diminished attention to the painful stimulus, and changing hemodynamics caused by a physiologic response to pain. Of these, the induction of descending pain control seems to be the most intuitive. Activation of the periaqueductal gray, *via* both descending connections from the hypothalamus, the amygdala, and the frontal lobe[33] and ascending connections from the dorsal horn of the spinal tract,[26] has been shown to produce

analgesia by reducing the activity of spinal cord neurons to painful stimulation. Such descending control could reduce pain signals coming into the brain, presumably decreasing the pain scale rating of the stimulation. However, this idea has not been supported in the literature as the cause of this BOLD contrast signal decay. Becerra *et al.*[5] and Apkarian *et al.*[34] both recorded pain scores immediately after each pain stimulus presentation. Neither found a significant change in their pain scale ratings across the stimulations, suggesting that habituation to pain is not the cause of the signal change attenuation. Pain scale ratings were not recorded after each stimulation in this study.

The data of this study indicate that nociceptor adaptation is not a likely cause. The transcutaneous electrical nerve stimulation used to invoke pain in this study activates all local nerves directly. This includes the A-β fibers responsible for sensation and the A-δ and C fibers responsible for pain transmission. If nociceptor desensitization was the cause, then signal change attenuation would not be present in a study that bypasses the nociceptor and directly activates the nerve. Because the nerve stimulation used here did result in attenuation, nociceptor adaptation does not seem to be a possible cause.

Attention to a painful stimulus has repeatedly been shown to affect brain activation. However, this affect has only reliably been shown in the insula and SII.[20,35] In addition, the activity in these areas typically shifts in location with attention and does not necessarily decrease in intensity. Because the signal decays witnessed here are strongly present in the ACC and SI, attention does not seem to be the cause of signal attenuation.

The effect of pain-induced changes in hemodynamics on the BOLD fMRI activation maps of pain is unclear because contradicting results have been presented. Global changes in cerebral blood flow (CBF) have been shown by both theory and experiment to alter the baseline MR signal and the BOLD signal.[36–38] Decreased CBF will decrease venous blood oxygenation and, as explained in the background section, will result in decreases in the overall MR signal. Becerra *et al.*[5] did not observe a drift in the unnormalized baseline MRI signal and concluded that global CBF does not change and does not cause the signal change attenuation. However, Coghill *et al.*[39] presented a positron emission tomography study that shows an overall decrease in global CBF caused by pain, which suggests that pain-induced decreases in global CBF could cause BOLD signal attenuation. Therefore, CBF is clearly a potential confounding factor that warrants further investigation.

In conclusion, this work provides clear scientific justification for the use of serial pain tasks in the fMRI evaluation of brain activation caused by painful ENS. It also confirms the signal change attenuation in brain areas activated by painful stimulation. Accounting for this in future data analysis may provide increased accuracy and highlights the importance of investigating the time course of fMRI data before applying standard analysis techniques. However, the cause of this attenuation is still unknown and provides another important aspect of pain processing in the brain that should be investigated.

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