Painful cutaneous laser stimuli induce event-related oscillatory EEG activities which are different from those induced by non-painful electrical stimuli.

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ABSTRACT

The non-phase locked EEG response to painful stimuli has usually been characterized as decreased oscillatory activity (event-related de-synchronization, ERD) in the alpha band. Increased activity (event-related synchronization, ERS) in the gamma band has been reported more recently. We have now tested the hypothesis that the non-phase locked responses to non-painful electrocutaneous stimuli are different from those to painful cutaneous laser stimuli when the baseline salience of the two stimuli is the same and the salience during the protocol is modulated by count laser and count electric tasks. Both of these stimuli were presented in random order in a single train at intensities which produced the same baseline salience in the same somatic location.

The response to the laser stimulus was characterized by five windows (designated Windows I to V) in the time-frequency domain: early (200-400 ms) and late (600-1400 ms) delta/theta ERS, 500-900 ms alpha ERD, 1200-1600 ms beta ERS (rebound), and 800-1200 ms gamma ERS. Similar ERS/ERD windows of activity were found for the electric stimulus. Individual participants very commonly had activity in windows consistent with the overall analysis. Linear regression of ERS/ERD for parietal channels was most commonly found for sensory- (pain or unpleasantness) or attention- (salience) related measures. Overall, the main effect for modality was found in Windows I – delta/theta and V - gamma, and the Modality with Task interaction was found in all five windows. All significant interaction terms included modality as a factor. Therefore, Modality was the most common factor explaining our results, which is consistent with our hypothesis.
Event-related changes in EEG spectral power can be measured by a decrease in oscillatory activity (ERD – event-related de-synchronization) or an increase in oscillatory activity (ERS – event-related synchronization) (34). These spectral responses (ERS/ERD) are not phase locked to the event but are analyzed by signal averaging in the frequency domain. Different frequency bands exhibit different temporal, spatial and task-related characteristics, which are consistent with their involvement in different aspects of cerebral processing (3; 5; 28; 51). Event-related spectral modulation of scalp EEG has also been applied to studies of the cortical processing of painful stimuli.

In response to painful stimuli, ERD has been found most frequently in the alpha band (10; 18; 23; 38; 47). ERS has also been reported at longer latency in the gamma band (12), or both the beta (rebound) and gamma band (21; 39). Some of these ERS/ERD activities have been associated with the perception of pain (10; 12) and with endogenous or exogenous attention to the painful stimulus (21; 23).

It is not clear that any one of these somatic sensory pain-related activities is specific for painful as opposed to non-painful somatic stimuli, or to the salience of the stimuli (24; 31). We have now tested the hypothesis that the non-phase locked responses to non-painful electrocutaneous stimuli are different from those to painful cutaneous laser stimuli when the baseline salience of the two stimuli is the same and the salience during the experimental protocol is modulated by count laser and count electric tasks. In these tasks, participants attended by counting either the painful laser stimuli (count laser) or the non-painful electrical stimuli (count electric) while both modalities of stimulation were presented in random order in a single train of stimuli, as in previous studies (7-9; 33; 52). The effect of directed attention toward or
distraction from a stimulus is to increase or decrease the salience of the stimulus relative to baseline (16; 31).

The present behavioral paradigm was focused on activities specific to pain. To control for the effect of somatotopic location of the stimulus, both painful laser and non-painful electrical stimuli were presented in the same somatic location (left dorsal forearm and hand). To control for the effect of expectancy, both stimuli were presented in random order and with random inter-stimulus intervals. To control for the effect of intrusiveness, both stimuli were presented at intensities which produced the same baseline salience, as determined for each participant in a baseline session prior to the experiment. Therefore, the protocol focused on ERD/ERS interactions specific to painful versus non-painful stimuli, as modulated by attention.

MATERIALS AND METHODS

Participants and EEG Recordings

Sixteen healthy participants (10 males and 6 females; aged 22-57 years) were recruited in this study. The protocol for this study was approved by the Institutional Review Board of the Johns Hopkins University School of Medicine; all participants signed an informed consent form prior to the study. The 19-channel EEG signals were recorded using Ag-AgCl electrodes (Grass) placed on the scalp according to the international 10-20 system with a referential montage to a reference of linked earlobes (Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T3, T4, Pz, P3, P4, T5, T6, O1, O2)(Jasper, 1958). Signals were amplified and digitized at the sampling rate of 500 Hz (SynAmps 5083, Neuroscan). The timing for the onset of the laser and electrical stimuli were acquired and digitally embedded in the recordings. The same data have previously been used to analyze causal functional interactions between signals recorded from different electrodes (37).
Experimental Design

Painful cutaneous laser and non-painful electrical pulses were delivered in four blocks of 80 stimuli (40 of each modality) in random order with random inter-stimulus intervals of 7 to 8 sec. The randomization procedures were carried out by the use of a standard random number generator (java.util.Random, Oracle, Redwood Shores, California).

In this study, attention was directed as participants were instructed prior to each block to count either the number of laser stimuli (count laser task) or the number of electrical stimuli (count electric task). The protocol consisted of two blocks of ‘count laser’ followed by two blocks of ‘count electric’, or vice versa. Therefore, there were a total of four blocks (Blocks 1, 2, 3 and 4), and the task of the first two blocks (Blocks 1 and 2) was randomly assigned to either the count laser task or count electric task, and counterbalanced across participants. The task of counting stimuli was the same whether attention was directed to the laser or the electrical stimulus. The experimental design of directing attention to one of the two stimulus modalities in a single train of stimuli have been used in studies of the attentional modulation of painful stimuli (7-9; 52). This protocol was designed to examine activity induced by painful and non-painful stimuli across a range of salience as manipulated by the experimental task, against a baseline salience that was equal for both stimuli.

Before the first block of stimuli, participants were not told that they would be asked to rate psychophysics of the stimuli at the end of the block. However, at the end of each block, participants were asked to report the number of attended stimuli in that block and to rate the pain intensity, pain unpleasantness, and salience of the laser stimulus. In addition, the participants were asked to rate unpleasantness and salience for the electrical stimulus. All the ratings in this study ranged from 0 to 10. The effect of priority upon these ratings is summarized in the Results section, based upon a detailed presentation of these results in an earlier publication (37).
Painful Laser and Non-painful Electrical Stimulation

The experiment was conducted in a silent, dimly lit room with the room temperature between 22 and 24 degrees Centigrade. Participants sat in a chair and rested their arms on a table in front of them. Insert earphones (ER1-14A Eartips, Etymotic Research, Inc, Elk Grove Village, IL) delivered a constant white noise (60 to 80 dB) throughout the experiment (Click-Tone control module, Astro-Med, Inc. GRASS Instrument Division).

Participants were asked to keep their eyes closed and sit quietly throughout the experiment while trains of painful laser and non-painful electrical stimuli were delivered. Both painful and non-painful stimuli were applied within the territory of the left superficial radial nerve. The non-painful electrical stimuli were constant current square-wave pulses of 1 ms duration and were delivered by a Grass S12 Isolated Biphasic Stimulator through skin electrodes (0.5 cm diameter, 1 cm inter-electrode distance) on the dorsum of the left wrist. The laser stimuli were generated and delivered using a Thulium YAG laser (Neurotest, Wavelight, Starnberg, Germany) with a laser-beam that had a wavelength of 2 µm, a diameter of 6 mm, and a duration of 1 ms. The laser stimuli were applied on the dorsum of the left hand. The stimuli locations were slightly different each time to avoid fatigue or sensitization.

At the end of each block, numerical rating scales were used to rate the participant’s psychophysical metrics regarding both painful and non-painful stimuli. For the non-painful electrical stimulus, unpleasantness was rated on a numerical rating which was anchored by 0 for the absence of unpleasantness and 10 for the greatest imaginable unpleasantness. For the painful laser stimulus, pain intensity and unpleasantness were rated separately on numerical rating scales. The pain intensity scale was anchored by 0 for the absence of pain and 10 for the maximum imaginable pain. For both modalities, salience was described as ‘the ability of the stimulus to capture attention’, and
was rated on a numerical rating scale for which 0 was the absence of salience and 10 was the most
salient stimulus imaginable.

For each participant, a series of laser pulses was delivered at 8 energy levels in an increasing
order ranging from 400 to 900 mJ, i.e. 400, 480, 560, 640, 720, 800, 850 and 900 mJ. Prior to the
experiment task, the participant was asked to rate the pain intensity for the given energy levels. The
laser energy for the experimental task was selected to be the level rated as a pain of 4-6 by the
participant. In addition, at each given energy level, the participant was asked to rate unpleasantness
and salience. The average energy level corresponding to a 4-6 pain intensity rating was 730 ± 170
mJ.

In a separate session prior to the experiment task, all participants were familiarized with
electrical stimuli by a series of electric pulses at intensity levels from 5.5 to 18 mA, all of which
were non-painful. Participants were asked to rate the unpleasantness and salience for each
intensity level. For each participant, the final intensity level of the electrical stimulus was
selected so that the electrical stimulus was non-painful and produced a salience rating which
was equal to the salience of the selected laser energy level (average current 12.8 ± 5.2 mA).

For each participant, both levels of electric and laser stimulation were fixed and applied during
all experiment so that at baseline, painful and non-painful stimuli were considered equally
salient.

**Event-Related Spectral Power (ERS/ERD ) Analysis**

In this study, the event-related spectral perturbation was used to estimate the event-related non phase-locked responses induced by the laser and electric stimulations (15). This
technique measures significant event-related changes in the power spectrum across different
frequency bands in the post-stimulus interval. In order to detect power changes across different
frequencies, each post-stimulus spectral estimate was divided by the mean baseline power spectrum and this ratio was the ERS/ERD.

Prior to the ERS/ERD analysis, the event-related potentials (ERPs) were estimated by averaging signals across trials and channels for each participant and task; these ERPs were subtracted from the signals (Figure 1, top row). In the statistical analysis of ERS/ERD data, we established the levels for significance (upper and lower boundaries) after the FFT application. We used a bootstrap procedure which was performed in over a 0.2 s time period immediately before the stimulus onset. The significant level was set to $\alpha=0.05$. The final results from this analysis were presented as a ratio between the baseline and post-stimulus estimates. It was called ERS if this ratio was larger than 1 and ERD if less than 1. The following provides a more detailed account of the statistical analysis of the power spectrum.

The EEG recordings were re-referenced to an averaged reference and filtered 0.1 to 250 Hz using a Hanning window finite impulse response filter. The event-related EEG epochs were extracted from every trial with a fixed interval of 0.5 s before and 2 s after at the onset of the stimulations. All EEG epochs were visually inspected by two independent individuals for artifact rejections. The time-frequency analysis was performed partly using newtimf.m in the EEGLAB, an open source toolbox, running in the 64-bit MATLAB (R2012a (7.14.0.739)) environment(15). Briefly, using the windowed FFT approach, the analysis estimated the significant event-related changes in the amplitude of the power spectrum across different times/frequencies. A sliding window with a length of 512 ms was used for the FFT and this window was advanced 5 ms throughout the whole epoch. The final time-frequency matrix for each epoch (about 80 epoch per subject per modality) was set to have 410 linear-spaced frequencies from 0.2 Hz to 100 Hz and 400 time stamps ranging from -244.0 to 1744.0 ms, and thus the resolutions for time and frequency were 0.24 Hz and 4.97 ms. To establish the levels of the significances (upper and
lower boundaries) after the windowed FFT application for each frequency step (from 0.2 to
100Hz, step size = 0.24 Hz), a bootstrap procedure was performed by randomly selecting the
spectral estimates in a shorter time period across epochs (i.e. 0.2 s immediately before the
stimulus onset) (15). The final results from this analysis were presented as a ratio between the
baseline and post-stimulus spectral estimates. It is called ERS if this ratio was larger than 1 and
ERD if less than 1.

RESULTS

Psychophysical Ratings

The psychophysics of the stimuli and the behavioral paradigm have been presented in a
preceding paper (37), and so are described briefly here. Across all participants, the painful laser
evoked sharp or pinprick pain sensations and the electrical stimulus produced a non-painful tingling
sensation. The pain intensity was significantly greater (5.4±2 vs 1.9±3; p<0.01, paired t-test) during
the count laser task than the count electric task. Error rates in this paradigm (total presented stimuli
minus reported stimuli) were low both for counting the total number of painful laser stimuli in the count
laser task, and for counting the non-painful electric stimuli in the count electric task (0.12±0.09 vs
0.10±0.12, P>0.05).

Stimulus intensities were adjusted so that both stimuli produced the same baseline salience
(prior to the task blocks); the average salience level for both the laser and the electrical stimulus was
5.3±1.9. The salience level for the laser stimulus during the count laser task (6.2±2.6) was not
significantly different from that for the electric stimulus during the count electric task (4.8±2.0, P>0.05
T-test). Similarly, the salience level for the laser stimulus under the count electric task was not
significantly different from that for the electric stimulus under the count laser task (2.0±1.4 vs
2.0±1.7, P>0.05). This was consistent with prior demonstrations that non-painful somatic stimuli can
be as salient as painful somatic stimuli (31; 40). Salience, pain (laser only) and unpleasantness
ratings were always higher for the stimulus which was counted in each task. Error rates were low, which indicated correct performance of the tasks overall.

To examine the effect of priority upon the salience ratings, we compared the first versus the second block in the first pair of blocks, and the first versus the second block in the second pair of blocks. The first pair blocks examined the laser stimulus under the count laser task, and the second pair of blocks examined the electric stimulus under the count electric task (see Methods). The order of the tasks between the first two blocks versus the second two blocks was randomized and counterbalanced across subjects. Overall, the results demonstrate that none of the differences in the ratings between the blocks of the same tasks and stimuli were significant (Table 2 in (37)).

ERS/ERD Spectral Windows: Laser versus electrical stimuli

The basis of all our analyses was time-frequency plots which were constructed for painful laser stimulation and non-painful electrical cutaneous stimulation (Figure 1A and 1B). The selection of windowing parameters (Table 1) was arbitrary, and was based upon the overall results (Figure 1) without accounting for differences in results between subjects, tasks or modalities. Therefore, the consistency between data overall versus individual subjects and factors was unlikely to be a product of windowing of the overall results. The color bar for the plot of the laser stimulus and count electric task was different from the other plots in order to optimally show activity in this category. Evidence of induced activity in this category (Figure 2 right upper) occurred in Windows I and II – delta/theta and channels Cz, Pz, C4 and P3.

The present ERS/ERD analysis was based upon results which were calculated with subtraction of the ERP, i.e. the top row of Figure 1. This analysis revealed patterns of ERS/ERD with five clearly distinct windows of induced activity, as shown in Figure 1. Windows I and II – both
delta/theta were the only windows showing significant phase locked activity (Figure 1, bottom row),
which indicated that activity in these windows reflected both evoked and induced activity. Other
windows showed only non-phase locked (induced) activity. Nevertheless, the time-frequency plots
did not show differences between induced power with and without subtraction of the evoked activity
(top and middle row of Figure 1), which suggested that the ERP did not influence the non-phase
locked time-frequency plot. The results of induced power with and without subtraction of ERPs and
evoked power were critical for the interpretation of ERS/ERD activity.

Time-frequency plots for both modalities of stimulation showed low frequency (delta theta
band) early windows (Table 1) that were bimodal with earlier and later windows (Figure 1A upper
row, Windows I and II – delta/theta). This was more evident in the count electric task for the
electrical stimulus (Figure 1B) and in the count laser task for the laser stimulus (Figure 1A). Both the
laser and the electric modalities showed an alpha ERD (Window III - alpha, Table 1) followed by a
beta rebound ERS (Window IV - beta), which was more pronounced in the count electric task for the
electrical stimulus and in the count laser task for the laser stimulus. The latency of these induced
low and high frequency non-phase locked responses were longer than those in previous studies (20;
38; 56). Some of these studies employed passive tasks and none employed the two stimulus
simultaneous presentation task of the present study, which was cognitively demanding and may
have led to longer latencies of activation.

These measures of ERS/ERD are all expressed as ratios of pre- to post-stimulus power (see
Methods: Event-Related Spectral Power Analysis). In order to give an indication of the raw power by
task modality category, we present the following median (95 – 5%ile) values across all windows and
channels: count laser – laser stimulus 17 (31 - 9) µV²/Hz, - electric stimulus 29 (48 -17); and count
electric – laser stimulus 18 (30 - 11), - electric stimulus 32 (57 - 18). These are consistent with the results for wakeful ongoing scalp EEG recording in healthy individuals (43).

ERS/ERD: Consistency between participants of ERS/ERD across EEG channels

We next examined the consistency of the results by tabulating the total number of subjects for which an ERS/ERD window was consistent with the same window and channel as in the overall analysis (Figure 1). This was expressed as the ratio of the number of subjects with consistent ERS/ERD channels by window and channel (see Table 2) divided by the total number of subjects (n=16).

As an example of this ratio, for the count laser task, Window V – gamma, and channel F3 15 out of 16 subjects had the same significant ERS/ERD as the overall analysis for a ratio of 15/16 or 0.94. This is termed as 1 miss since one channel did not have the same significant ERS/ERD as the overall analysis. For the count laser task (Table 2 - upper), each window within channel had ratios of 1 to 0.68. For the count electric task, the ratios were between 1 and 0.5. This analysis was an indicator of the generalizability of the present results, which will be a basis for comparisons with other studies.

These results demonstrate a congruent pattern of ERS/ERD between individual participants versus the overall analysis. Table 2 shows that most participants had no misses for the majority of the window channel pairs. This might suggest that those windows for which ERS/ERD in individual participants were different from the overall analysis occurred equally among all participants. Therefore, we examined the results by looking for misses by channel and window combinations within individuals.
The windows that were different from the overall analysis were mostly in Window III - alpha.

These Window III - alpha misses were found in participants 1, 5, 9, 11, 12, and 15 who were missing activity in 1, 3, 5, 3, 3, and 5 channels, respectively. The windows III - alpha with missing ERS/ERC results (Table 2) were largely found in channels C3, C4, Cz, P3, P4 and Pz. The frequent absence of activity in Window III - alpha may be reflected by the variability of between channels in average time-frequency plots, such as count laser task - laser stimulus (Figure 2 upper left). However, in the case of Window I - delta/theta, only one channel lacked ERS, i.e. participant 3. Such misses were infrequent for the other windows, as shown in Table 2. Overall, the number of misses for Window III – alpha were significantly greater than those for Window I – delta/theta (6/16 vs 1/16, $P=0.041$, Fisher) and any other window (6/15 vs 0/16, $P=0.009$).

The consistency of activations in windows across subjects suggests that the results of the overall analysis will be generalizable. In the case of Window III – alpha, five subjects had misses at multiple channels which suggested that a population of subjects was characterized by common misses in this window. However, subjects with multiple channels missing Window III – alpha showed no differences versus other subjects in ratings of task related pain and unpleasantness, or error rates or laser power settings.

Laser ERS/ERD: Effect of Stimulus, Task and Channel within Window

We next undertook an ANOVA model of ERS/ERD with Channel, Task and Modality factors, for each window. The F3, F4, C3, C4, Cz, P3, P4, and Pz channels for each subject were selected for the analysis of the ERS and ERD (23; 38; 44). For each time-frequency window, the ERS/ERD results were modeled by the three factors in a within subject analysis of variance (ANOVA; 2 Tasks x 2 Modalities x 8 Channels). The Wilcoxon signed rank test was
used for the post-hoc analysis and the significance level was set at p<0.05 with Bonferroni correction for multiple comparisons.

In Window I - delta/theta, Modality and Channel were the main effects (p=0.02 for Modality and p<0.00001 for Channel), and there were significant interactions of Task with Modality (F_{1,15}=5.92, P<0.03), and of Task with Channel with Modality (F_{7,105}=2.34, p=0.03). Post-hoc testing revealed that the Cz channel had significantly larger ERS responses in the count laser than the count electric task for the laser modality (Table 3), which was reflected in Figure 2 (upper left dense red versus upper right yellow). The interaction terms were reflected in F3, F4, C3, C4, Cz, and Pz, which were found to have significantly larger ERS for the electric stimulus in the count laser task than laser ERS in the count electric task (Table 3, Wilcoxon signed rank test, p<0.0063). This was reflected in Figure 2 (bottom left (dense red) versus top right (light colors)) in the Window I - delta/theta ERS levels for all channels except for P3 and P4 (Wilcoxon signed rank test, p<0.0063).

- Place Figure 2 about here –

For Window II - delta/theta, Task and Channel were the main effects (p=0.026 and p<0.0003 respectively) and there was significant interaction of Task with Modality (F_{1,15}=10.80, p=0.005). Post-hoc testing revealed that F4 and Cz had significantly larger ERS activity in the count laser than the count electric task for laser modality, as summarized in Table 3 (Wilcoxon signed rank test, p<0.0063). This was reflected in the laser modality for the count laser task (Figure 2, upper left dense red) compared to the count electric (Figure 2, upper right light colors).

- Place Table 3 about here -

In Window III - alpha, Channel was a main effect (p=0.003) and there were significant interactions of Task with Modality (F_{1,15}=19.52, p=0.005), and of Task with Modality with Channel (F_{7,105}=2.538, p=0.02). Post-hoc testing revealed that C3 had significantly larger ERD
responses in the count laser than the count electric task for the laser modality (Table III). This
was reflected in the laser modality for the count laser (Figure 2, upper left, intense blue) versus
count electric task (Figure 2, upper right, yellow green), which may be related to the small ERD
variability for this pair. The high mean ERD values for Cz, Pz and P4 were influenced by the
variability of a population of subjects having multiple misses in Window III particularly in
channels C3, C4, Cz, P3, P4 and Pz (see Section ERS/ERD: Consistency ..., Table 2).

In Window IV - beta, no main effect was found but a significant interaction of Task with
Modality was found in this window ($F_{1,15}=8.934$, $p=0.01$). However, the post-hoc test showed no
significant result after correction for multiple comparisons. This was reflected in the yellow to
red Window IV - beta activity which was found in all four modality and task displays in Figure 2.

In Window V - gamma, the Modality was a main effect ($p=0.04$) and there was a
significant interaction of Task with Modality ($F_{1,15}=16.64$, $p=0.001$). Post-hoc testing revealed
that C3 had greater laser-ERS in the count electric task (Figure 2, upper right, yellow and red
patchwork) than electric-ERS in the count laser task (Figure 2, lower left, solid blue) (Wilcoxon
signed rank test, $p=0.0011$).

Overall, the interaction by modality with task was the only effect which was found in all
windows in this statistical analysis, and which was reflected by the contrasts in Figure 2.
Modality was a main effect in Windows 1 – delta/theta and V – gamma, and all interactions
included Modality as a factor. These results indicate that modality was an important component
in the model of 2 Tasks x 2 Modalities x 8 Channels.

Regression of Laser Pain Psychophysics versus ERD/ERS by Channel

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The overall changes for psychophysical ratings (post-stimulus minus baseline pre-stimulus) and task error rates for the laser stimulus were correlated with the ERS or ERD as appropriate to the window (e.g. Window III – alpha ERD) and the tasks by linear regression analysis (p<0.05). All significant results showed linear regression with a positive slope, as indicated in Table 4 by the + sign following the channel at which significant regression was found.

For parietal channels across several windows (P3, P4, Pz), sensory (pain or unpleasantness) or attention (salience) ratings most commonly had significant linear regression on ERS/ERD. Specifically, the P3 and P4 electrodes had significant regression more commonly than all other electrodes (11/15 vs 4/15, P=0.027, Fisher). A relationship between electrodes with ERS/ERD activation was not more common at parietal channels for sensory and attention measures versus task error rates (7/15 vs 4/15, P=1). These results suggest that parietal structures may have a disproportionate role in ratings of sensory and attention.

DISCUSSION

Overall, the main effect for Modality was found for Windows I – delta/theta and V – gamma, and the Modality with Task interaction was found for all five windows. Task main effects were found for Window II - delta/theta and Task with Modality interactions were found for all windows, as above. Channel was a main effect for Windows I – delta/theta, II – delta/theta, and III - alpha, and there were no interactions of channel with Modality or Task. However, Channel with Task with Modality interactions were found for Windows I – delta/theta and III - alpha. There was no significant interaction term that did not include Modality as a factor.
Therefore, the Modality dependent main effects, and the interactions of Modality with Task, and of Task with Modality with Channel together were the most common effects explaining these results. These results are consistent with our hypothesis that the non-phase locked responses to the non-painful electro-cutaneous stimulus modality are different from those to the painful laser modality.

Many of the latency differences between windows for the laser and electrical modalities may reflect the longer laser receptor utilization time and transmission delay of the Adelta and C fibers versus the Abeta fibers, which are activated by the electrical cutaneous stimulus (25; 26; 53). These later onset latencies are evident for the laser versus the electrical Modality in Figure 1 Window I – delta/theta, as well as the alpha ERD and the beta ERS rebound (Figure 1, Windows III - alpha and IV - beta). Later offset latencies are found at Windows II – delta/theta, III – alpha, and IV - beta of the laser versus the electrical stimulus, and may also reflect processes which occur after transduction and transmission of the afferent signal.

The time-frequency plots of ERD/ERS activity are similar to previous reports that phasic painful stimuli produce non-phase locked alpha ERD alone (10; 23; 38; 47), or gamma alone (12), or alpha ERD followed by beta ERS rebound (21; 24). These findings are consistent with another study of changes in non-phase locked responses to phasic pain stimuli (21). The same pattern of alpha ERD followed by beta ERS rebound is reported with somatic sensory stimuli such as electrical stimulation of the median nerve (4; 49), with different motor tasks (41; 42), and with electrical cutaneous stimuli, as in the present case.

Psychophysical protocols can exert powerful influences upon evoked ERS/ERD activity. Several studies have examined the effect of directed attention in an oddball protocol upon magneto-encephalographic (MEG) sources activated by a painful intra-cutaneous electrical stimulus. When count laser was directed toward one stimulus, then a MEG alpha ERD (present...
Window III - alpha) was recorded (23)(see also (38)), which is consistent with the effect of count laser upon Window III - alpha ERD (Figure 1, top row). Early gamma ERS (present Window V - gamma) was greater with directed attention to the high intensity target (oddball) stimulus (21).

These results suggest that attention to a phasic painful stimulus induces alpha ERD and gamma ERS, which is consistent with the present results.

ERD/ERS versus Pain Perception

The present results show evidence of correlation between ERD/ERS activity in all five windows and pain perception. This is consistent with a study that examined responses to three laser stimuli in a short train. That study reported that pain intensity of the first stimulus was correlated with ERD and ERS (24), although alpha ERD was not correlated with pain intensity. Direct correlations with pain perception were also reported in the case of ‘prefrontal’ gamma power (12), and in the case of three alpha EEG sub-bands that showed ERD with the anticipation of pain (10).

The early gamma ERS found at channel C4 during the count electric and laser tasks for the laser modality (Figure 2) might be consistent with that reported in SI of a prior study (56), based on visual comparison. In the present results higher pain ratings were found for the count laser task versus the count electric task and the C4 early gamma was correspondingly larger (Figure 2). The early gamma ERS found at the F4 channel for the laser modality and the count electric task might reflect the cognitive aspect for the pre-frontal lobe regulation of pain (11; 54), consistent with reference (12).

The identity of structures in the brain generating the present scalp EEG results must be interpreted cautiously. Nevertheless, these results seem to be consistent with the correlation
between stimulus intensity and pain rating in a study of alpha ERD carried out through subdural electrodes (44). In that report, alpha ERD was maximal and more widespread over primary somatosensory cortex and parasylvian cortex during directed attention to the painful laser stimulus (counting stimuli) versus distraction (reading for comprehension)(44). The relationship between pain perception and neural recordings has also been explored through analysis of the intracranial recordings of human thalamic neuronal firing and cortical local field potentials (29; 32; 45). The relationship of ERD/ERS activity to pain perception and attention is strong evidence of the relationship between this activity and pain sensations.

Methodological Considerations

In order to study the changes for the non-phase locked activities precisely, it is important to determine that there are minimal phase locked components in the overall power spectrum. This is accomplished by subtracting the ERP from every epoch to eliminate phase-locked components and to retain only non-phase locked signals (Figure 1)(2; 50). The difference between the ERS/ERD with subtraction versus ERS/ERD without subtraction has been both predicted and observed to be very small relative to the ongoing EEG (36)(see also Figure 1). However, the non-phase locked activities cannot be precisely discriminated when both (phase and non-phase) activities are present within the same window. Furthermore, individual channels do not always reflect the overall analysis, which is described above (Table 2) and which may reflect the non-phase locked activity described by (56). Therefore, early latency ERS found in this study might originate to some degree from both phase locked and non-phase locked activity in Windows I and II – delta/theta (2; 27). From a practical point of view, the ERP subtraction procedure has been widely used for both EEG and MEG signals to investigate the induced non-phase locked activities in humans (13; 21; 50).
Salience is a property of painful and non-painful somatic sensory stimuli, and of stimuli of special sense, e.g. vision (16; 17; 40). The response to the painful cutaneous laser may reflect the salience of the stimulus, defined as ‘the ability of the stimulus to capture attention’ (40)(see also (16; 17; 30; 35; 55)). We controlled for the effect of baseline salience of the two stimuli within each participant by matching the salience of the electric stimulus to that of the painful laser stimulus through adjustments in the intensity of the stimuli. Salience of a stimulus was altered by task so that salience was not equal under different tasks. In addition, both stimuli were applied in the same somatotopic location. This method was designed so that the presence or absence of pain was the main difference between the two stimuli. The present results are an example of ‘the usefulness of laser-related activity … to explore the effect of a given experimental factor on the transmission and processing of nociceptive input’ (40).

It could be suggested that the consistency of these results is a function of the windows selected for this analysis (Table 1). Time-frequency plots were constructed for painful laser stimuli and non-painful electrical cutaneous stimuli (Figure 1A and 1B). The selection of windowing parameters (Table 1) was descriptive, and was based upon the overall results (Figure 1) without accounting for differences in results between subjects, Tasks or Modalities. Therefore, the consistency between data overall versus individual subjects and factors is unlikely to be a product of windowing of the overall results.

Possible Generators for ERD/RES Non-Phase Locked Activity

In the present results, maxima during the count laser task were found in central electrodes for Windows I and II – delta/theta, and III - alpha, and during the count electric task for Window V – gamma (Table 3). Consistent with this finding are a number of studies in which alpha ERD was found at C4 and Cz (38), or over sensorimotor channels (47), or over contralateral central channels (23).
These results seem to be consistent with MEG studies which generate a 3D estimate of the generator of activations. In the case of MEG recordings, delta ERD was maximal over sensorimotor channels, alpha ERD over sensorimotor structures (21), and beta ERD over primary motor cortex (48).

Functional imaging studies also reflect the possible generators of the non-phase locked ERS/ERD activity reported here.

The anatomical relationship of non-phase locked EEG to functional imaging techniques is complex. PET and fMRI studies have demonstrated modulation of blood flow or BOLD in the primary (SI) and secondary somatosensory cortices (SII), anterior cingulate cortex (ACC), orbitofrontal cortex, and insula by attention to painful stimuli (1; 6; 14; 19; 22; 46). These findings may be consistent with the significant ERS at central and midline channels during the count laser task (Table 3). Distraction produced decreased activations in SI, SII and insula (6; 46), which may be consistent with decreased ERS/ERD over central channels.

ERD/ERS and ERC

In a prior study the majority of individual participants had ERC in the same channel as classified by task and direction of functional interaction as in the overall analysis (37). Similarly, the majority of individual participants had ERS/ERD activity in the same channels as in the analysis overall. These results point to substantial consistency across participants for causality and activation. Channels with ERC were significantly more common during count laser Task, and Channels with ERD/ERS were more common during the count laser than during the count electric Task. These findings raise the possibility that ERC is related to ERD/ERS as a function of Task.
**FIGURE LEGENDS**

**Figure 1**: Time-frequency plots of ERS/ERD in the count laser and count electric Tasks as averaged across all participants and channels for the response to the painful laser stimulus (A) and the non-painful electrical stimulus (B), as labeled. The top row and the middle row represent these plots with and without subtraction of the laser-evoked potential (A as labeled) and electrical cutaneous-evoked potential (B as labeled). The bottom row indicates the phase locked time-frequency plot evoked by the laser (A) and the electrical cutaneous stimulus (B). In all rows, hot and cold colors indicate increases and decreases in power relative to the background level. For example, 1.5 and 0.5 in the color bar scale denote 1.5 and 0.5 times the baseline power, and therefore the ERS and ERD. 1 denotes the baseline power. The color bar for the response evoked by the laser stimulus in the count electric plot (Figure 1A) is different from the other plots in order to show activity in this category more clearly.

**Figure 2**: Time-frequency plots of ERS/ERD in all four Tasks by Modality combinations as averaged across all participants and within channels, as labelled in the upper left panel (Attention Task, Laser Modality). Conventions for axes and color scales are as described in the legend for Figure 1 and as shown in the upper left panel of this figure.


<table>
<thead>
<tr>
<th></th>
<th>Window I – delta/theta</th>
<th>Window II – delta/theta</th>
<th>Window III - alpha</th>
<th>Window IV - beta</th>
<th>Window V - gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERD/ERS Laser Time</td>
<td>200-400ms</td>
<td>600-1400ms</td>
<td>500-900ms</td>
<td>1200-1600ms</td>
<td>800-1200ms</td>
</tr>
<tr>
<td>ERD/ERS Laser Frequency</td>
<td>0-8 Hz</td>
<td>0-8 Hz</td>
<td>8-10 Hz</td>
<td>15-25Hz</td>
<td>40-50Hz</td>
</tr>
<tr>
<td>ERD/ERS Electric time</td>
<td>150-500ms</td>
<td>700-900ms</td>
<td>400-550ms</td>
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<td>ERD/ERS Electric frequency</td>
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<td>8-10 Hz</td>
<td>10-20Hz</td>
<td>40-50Hz</td>
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**Table 1**: Time and Frequency dimensions for the Windows for ERS/ERD.
<table>
<thead>
<tr>
<th>Window</th>
<th>F3</th>
<th>F4</th>
<th>C3</th>
<th>C4</th>
<th>Cz</th>
<th>P3</th>
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<td>1</td>
<td>1</td>
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<tr>
<td>V</td>
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<td>1</td>
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<td>1</td>
<td>0.94</td>
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<table>
<thead>
<tr>
<th>Window</th>
<th>F3</th>
<th>F4</th>
<th>C3</th>
<th>C4</th>
<th>Cz</th>
<th>P3</th>
<th>P4</th>
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<tr>
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<td>0.81</td>
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<tr>
<td>II</td>
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<td>0.94</td>
<td>0.88</td>
<td>1</td>
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<tr>
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<td>V</td>
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<td>1</td>
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<td>1</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Consistency of a significant change in ERS/ERD in a window across individual subjects versus the window overall. If all subjects had significance in the same window as the overall analysis then the consistency had a value of 1; if no subject had a significant window then the consistency had a value of 0.
Table 3: The channels with significantly higher ERS/ERD by Window, Task and Modality as determined in the post hoc tests of the ANOVA described in the text. * indicates greater ERS/ERD activity for the task with the * versus the other task, while both tasks are under the same modality. For example, in Window I – delta/theta under the laser modality and attention task the * by Cz indicates that the Cz has greater ERS for the attention than the distraction task while the laser modality remains the same. † indicates greater ERS/ERD for the modality with the † versus the other modality, while the task remains the same. *† indicates greater ERS/ERD for the modality and task of the channel with the *† versus both the other modality and task, i.e. interaction term. For example, in window I – delta/theta and channel F3 the electric stimulus and the count electric task had the larger ERS than the laser stimulus and the attention task.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Task</th>
<th>Modality</th>
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<tr>
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<td>Laser</td>
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<tr>
<td></td>
<td></td>
<td>Electric</td>
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<td>Cz*</td>
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<tr>
<td></td>
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<td>F3†, F4†, C3†, C4†, Cz†, Pz†.</td>
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<td>F4* Cz†</td>
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<tr>
<td>Window V - Gamma</td>
<td>Count Laser</td>
<td>C3†</td>
</tr>
<tr>
<td></td>
<td>Count Electric</td>
<td></td>
</tr>
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Table 3: The channels with significantly higher ERS/ERD by Window, Task and Modality as determined in the post hoc tests of the ANOVA described in the text. * indicates greater ERS/ERD activity for the task with the * versus the other task, while both tasks are under the same modality. For example, in Window I – delta/theta under the laser modality and attention task the * by Cz indicates that the Cz has greater ERS for the attention than the distraction task while the laser modality remains the same. † indicates greater ERS/ERD for the modality with the † versus the other modality, while the task remains the same. *† indicates greater ERS/ERD for the modality and task of the channel with the *† versus both the other modality and task, i.e. interaction term. For example, in window I – delta/theta and channel F3 the electric stimulus and the count electric task had the larger ERS than the laser stimulus and the attention task.
<table>
<thead>
<tr>
<th>Window</th>
<th>EEG change</th>
<th>Task</th>
<th>Psychophysics</th>
<th>Channel</th>
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<tr>
<td>I – Delta/theta</td>
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<td>Count electric</td>
<td>Task Error Score</td>
<td>P3+, P4+</td>
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<td>Count laser</td>
<td>Task Error Score</td>
<td>C3+, P3+</td>
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<td></td>
<td>Count electric</td>
<td>Pain Rating</td>
<td>P3+, P4+, Pz+</td>
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<td>Pz+</td>
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<td>IV – Beta</td>
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<td>Unpleasantness</td>
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<tr>
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<td>Salience</td>
<td>Pz+</td>
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<tr>
<td>V - Gamma</td>
<td>ERS</td>
<td>Count laser</td>
<td>Salience</td>
<td>P4+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Count electric</td>
<td>Pain Rating</td>
<td>Pz+</td>
</tr>
</tbody>
</table>

**Table 4**: The channels with significant linear regression results of ERD/ ERS versus psychophysical measures in the laser modality.