Electrophysiological studies on pain-evoked neuronal responses are crucial for a better understanding of pain perception mechanisms in humans. Noxious stimulation with short-pulse laser was introduced to pain research around three decades ago. Unlike electric stimulation, laser stimulation selectively activates cutaneous nociceptive receptors without simultaneously eliciting a tactile response. Thus the peripheral A-delta (Aδ) and C fibers are involved in the generation of the resultant laser-evoked potential (LEP) and magnetic field (LEF) recorded by electroencephalography (EEG) and magnetoencephalography (MEG), respectively. With a better spatial resolution, MEG is more suitable than EEG for studying pain processing. Previous time-domain analyses of pain-elicited brain responses have shown the involvement of complex cortical networks including the primary (SI) and secondary somatosensory (SII) cortices, the insular cortex, the anterior cingulate cortex, and the dorsolateral prefrontal cortex. However, the frequency characteristics of pain-evoked responses remain unclear.

The information obtained with either EEG or MEG reflects an ensemble of neuronal sources that generate oscillatory

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activities in various frequency ranges. Responding to adequate peripheral stimulation, these sources are coherently activated and coupled with resultant change in brain rhythms.14,15 Previous studies have reported the participation of delta to gamma bands in the visual and auditory evoked activations.14-22 Recent EGG studies have shown frequency-specific over-activations in patients with neurogenic pain, which suggests a role of the thalamocortical loop in pain processing.23,24 Moreover, Hauck and coworkers have reported an involvement of high-frequency activity in the cerebral mechanisms of attentional augmentation of pain processing.25 Therefore, it is worthy further investigating the oscillatory dynamics of pain-evoked brain responses.

Most biosignals that vary around a mean value can be reconstructed as a sum of sine and cosine waves occurring at different frequencies.26 The spectral wavelet analysis allows the overall variance of a biosignal to be split into individual frequency components.26 Power difference between various oscillations might be attributed to differential involvement in pain processing. We therefore hypothesized that some oscillations might specifically reflect the correlation between SI1 activation and subjective pain rating. In this study, we identified the oscillatory components of laser-evoked MEG responses by using wavelet transform analysis, and then compared with their power. To explore this hypothesis, this study involves: a) documenting the quantitative changes of pain-related oscillatory activities, b) analyzing the correlations between perceived pain magnitude and the oscillatory activities of the SI2 cortex, and c) tracking the cortical representations of pain-related oscillatory activities.

**METHODS**

**Subjects**

Ten healthy volunteers (eight men and two women; mean age 32.1 ± 4.3 years; all right-handed) were recruited to participate in this study. None had any neurological or psychiatric deficits. Each subject gave their informed consent prior to the experiment. Our study protocol was approved by the institutional review board of Taipei Veterans General Hospital.

**Laser pulse stimulation and pain rating**

Cutaneous nociceptive stimuli were produced using a thulium-YAG laser stimulator (BLM 1000 Tm:YAG®, Baasel Lasertech, Starnberg, Germany) set up in the MEG lab at Taipei Veterans General Hospital. The laser emits near-infrared radiation with a wavelength of 1.96 μm, a spot area of 10 mm², and a pulse duration of 1 ms; resulting in a penetration depth of 360 μm into the human skin. This laser beam was then conducted via an optical fiber, into a magnetically shielded room through a small hole. The stimuli were applied to the lateral dorsum of the left hands of the volunteers by an assistant who held the hand-piece at the end of the optical fibre and kept the stimulator head stably placed on the skin. In order to avoid skin burns and fatigue of the primary nociceptive afferents, our research assistant slightly changed the position of hand piece within an area of 3-4 cm in diameter following each stimulus. To find the three different laser pulse intensities rated by each subject as mild, moderate and severe pain respectively, we asked all the subjects to rate a train of laser pulse stimulations starting from 100 mJ and increasing in 50 mJ steps. Each subject was instructed to rate the perceived intensity of a stabbing pain using the Visual Analogue Scale (VAS).27,28 We determined pain threshold as the lowest intensity level that evoked clear stabbing pain (VAS = 1). The VAS 0 was defined as no pain, and VAS 10 as the worst imaginable pain. We determined the lowest strengths of laser pulses for eliciting pain levels at VAS 2-3, VAS 5-6, VAS 8-9 for each subject, and then applied the stimuli on each subject to elicit mild, moderate and severe pain, respectively. The above methodology for stimulation and pain rating has been detailed elsewhere.29 Accordingly, the stimulus intensities for producing mild, moderate, and severe pain were determined to be 255, 365, and 490 mJ, respectively, when averaged across all subjects.

**MEG measurement**

During the MEG recordings, each subject sat comfortably in a magnetically shielded room with the head supported against the helmet-shaped bottom of a whole-scalp 306-channel neuromagnetometer (Vectorview™, Elekta Neuromag, Helsinki, Finland). Our neuromagnetometer comprised 102 identical triple sensor elements, and each sensor element consisted of one magnetometer and two orthogonal planar gradiometers. In the present study, the data analysis was based on the signals of the 204 planar gradiometers, because of relatively poor signal-to-noise ratio for magnetometer signals.29

Each subject underwent three sessions (mild, moderate, and severe pain) of laser pulse stimulation in a randomized order. Before each session, the subject had a five to ten minute rest. Forty responses were averaged in each session. The interstimulus interval (ISI) varied between 8 and 12 s. The signals were band-pass filtered (0.1-160 Hz) and digitized at 500 Hz. Epochs were excluded from being averaged whenever the amplitudes of the corresponding electro-oculogram and MEG signals were larger than 300 μV and 6000 fT/cm, respectively.

The exact location of the head with respect to the sensors was found by measuring magnetic signals produced by currents that were led to four head indicator coils, placed at known sites on the scalp. The locations of the coils with respect to anatomical landmarks on the head were determined with a three-dimensional (3-D) digitizer to allow alignment of the MEG and magnetic resonance (MR) image coordinate systems.29 The MR images of the brain of each of the subjects were acquired with a 3 T Bruker Medspec300 scanner (Germany).

**Wavelet analyses and equivalent current dipole (ECD) modeling**

The LEF responses of the gradiometer channels were computed with the continuous wavelet transform by using MATLAB 6.5 programming software (The MathWorks, Natick, MA, USA). The analysis period of 1100 ms included a prestimulus baseline of 100 ms. The Morlet wavelet30 is a function of time t and frequency f0 defined as:

\[ w(t, f_0) = A \exp(-t^2/(2\sigma_t^2)) \exp(2\pi i f_0 t), \]

where \( \sigma_t = 1/(2\pi\sigma_f) \) and \( A = 1/(2\pi\sigma_f^2)^{1/2} \).

The width of the wavelet (m = \( f_0/\sigma_t \)) was chosen to be 7.31-38 The time-varying amplitude of the neuromagnetic responses in a frequency band around \( f_0 \) is the result of the convolution of the complex wavelet \( w(t, f_0) \) with the signal s(t) :
This procedure was performed by using a set of wavelets with $f_0$ ranging from 0.5 to 25 Hz at intervals of 0.5 Hz.

Time-frequency representation of pain-related responses was obtained from the squared norm of $E(t, f_0)$ with $f_0$ ranging from 0.5 to 25 Hz in all channels. The spatial distribution, power and temporal features of the stimulus-related oscillatory activities were exhibited. To see the oscillatory characteristics following laser stimulation, we inspected the pain-related time-frequency representations, and selected the single channel with maximal oscillatory activities located around the SII from both hemispheres for further analysis. The time-frequency plots for the selected channels were averaged across individual frequency bands of 0.5–4 Hz, 4–8 Hz, 8–13 Hz and 13–25 Hz to provide the time-varying measures of delta, theta, alpha and beta activities, respectively. The mean power value during the 100 ms prior to stimulus onset was considered as the baseline level and was subtracted from the power after the stimulus onset. Peak latencies were derived from the time point of maximal power for individual rhythmic activities.

We further averaged the $E(t, f_0)$ of all the channels that related to the bands of interest mentioned above and obtained the amplitude fluctuations of the rhythmic activities. The oscillatory activities were then modeled with ECD modeling. To obtain the activation areas, we visually searched the oscillatory deflections that clearly exceeded the prestimulus background level. The single ECD that best described the measured data was found by a least-squares search using the subsets of 24-30 channels around the maximal responses. Goodness-of-fit of the model was calculated and only ECDs explaining more than 80% of the field variance at selected periods of time over the subset of channels were used for further analysis. These calculations resulted in the 3-D location and orientation of the ECD in a spherical conductor, which were coregistered with the MR images of each subject’s brain. The positive x-, y-, and z-axes in our head-coordinate system were set towards the right preauricular point, the nasion, and the head vertex, respectively.

**Statistics**

In the present study, we evaluated the peak powers, latencies and ECD locations with respect to the effects of pain intensity (mild, moderate and severe), frequency bands (delta, theta, alpha and beta), and the hemisphere (contra- and ipsi-lateral SII) by using non-parametric repeated measures ANOVA (Friedman ANOVA). When a significant effect was found, post-hoc comparisons were performed by using Wilcoxon’s signed ranks test. The significance threshold was taken as $p < 0.05$.

**RESULTS**

**Time-frequency representations**

Figure 1(A) shows the time-frequency representation of the evoked neuromagnetic responses of Subject 1 by moderate painful stimulation. It shows the spatial distribution of 0.5–25 Hz activities 100 ms before and 1000 ms after stimulus onset. The enhanced oscillatory activities are clearly discernible in the bilateral temporoparietal areas. Figure 1(B) displays the time-frequency plots from the two channels of interest located around the bilateral SII of Subject 1 in response to mild, moderate, and severe pain conditions. L, left; R, right.
severe painful stimuli. A power increase was found in all conditions around 150-220 ms following stimulus onset, with maximal power ranging between 5 and 10 Hz. Notably Subject 1 additionally shows long-lasting power suppression in the theta range, occurring around 500 ms after stimulus onset and lasting up to 1000 ms. This finding was not, however, included in our analysis because of its inconsistency across subjects.

**Pain-related oscillations**

Figure 2 shows the power (mean ± SEM) of the delta to beta frequency bands from the two channels of interest in the bilateral hemispheres in response to mild, moderate and severe pain. We observed a significant bilateral power increase in the delta, theta and alpha activities to all the three pain intensities (all $p < 0.01$). As for the factor of frequency band, statistical analysis showed significant differences (all $\chi^2 > 12$, all $p < 0.01$). Post hoc comparison revealed that both the theta and alpha activities in all pain intensity conditions, as well as in the bilateral hemispheres, were significantly larger than the delta and beta activities ($p < 0.01$), which indicated that the pain-related oscillatory activities take place predominantly within the theta and alpha frequency ranges. With respect to the hemispheres, no significant power difference was found in any of the pain intensity conditions (all $p > 0.2$). Finally, pain intensity elicited significant differences in the theta ($\chi^2 = 7.8$, $p < 0.05$) and alpha ($\chi^2 = 9.8$, $p < 0.01$) activities in the bilateral hemispheres. Wilcoxon testing showed that the power of the theta and alpha bands under mild painful stimulation were significantly smaller than when moderate and severe pain stimuli were used (for theta, $p < 0.02$; for alpha, $p < 0.05$). However, bilateral theta and alpha activities were comparable in power in moderate and severe pain conditions (all $p > 0.1$).

The Table below summarizes the peak latency of delta to beta activities for different pain intensity conditions. The overall mean (± SEM) latencies across all conditions was 194 ± 2.4 ms. Statistical analysis of the peak latencies showed no significant difference with respect to the frequency band ($\chi^2 < 5$, $p > 0.1$), hemisphere ($\chi^2 < 3$, $p > 0.1$), and pain intensity ($\chi^2 < 4$, $p > 0.1$).

<table>
<thead>
<tr>
<th>Pain condition</th>
<th>Band</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mild</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>delta</td>
<td>209 ± 10</td>
<td>206 ± 15</td>
<td></td>
</tr>
<tr>
<td>theta</td>
<td>210 ± 10</td>
<td>197 ± 7</td>
<td></td>
</tr>
<tr>
<td>alpha</td>
<td>206 ± 13</td>
<td>189 ± 14</td>
<td></td>
</tr>
<tr>
<td>beta</td>
<td>206 ± 13</td>
<td>189 ± 11</td>
<td></td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>delta</td>
<td>209 ± 14</td>
<td>201 ± 14</td>
<td></td>
</tr>
<tr>
<td>theta</td>
<td>187 ± 11</td>
<td>206 ± 8</td>
<td></td>
</tr>
<tr>
<td>alpha</td>
<td>194 ± 13</td>
<td>182 ± 9</td>
<td></td>
</tr>
<tr>
<td>beta</td>
<td>182 ± 13</td>
<td>183 ± 10</td>
<td></td>
</tr>
<tr>
<td><strong>Severe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>delta</td>
<td>190 ± 13</td>
<td>206 ± 15</td>
<td></td>
</tr>
<tr>
<td>theta</td>
<td>198 ± 8</td>
<td>198 ± 7</td>
<td></td>
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<tr>
<td>alpha</td>
<td>182 ± 12</td>
<td>186 ± 9</td>
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<tr>
<td>beta</td>
<td>207 ± 11</td>
<td>183 ± 10</td>
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</tr>
</tbody>
</table>

**Cortical localization of pain-related oscillations**

Figures 3(A) and 3(B) show the spatial distributions of time-varying theta and alpha activities for moderate painful stimulation from Subject 1 and Subject 2, respectively. Clear response deflections are observed in the bilateral temporoparietal areas. For theta and alpha activity, one in the ipsilateral hemisphere peaks at 192 and 184 ms; another one in the contralateral hemisphere at 186 and 182 ms, respectively. The isocontour map suggests the presence of a single ECD in each hemisphere, which are localized in the superior bank of the Sylvian fissure, around the SII area.

Figure 4(A) shows the ECDs of theta and alpha activities of Subject 2 in various pain stimulus conditions superimposed on his own MR images. These ECDs were localized in the vicinity of the superior bank of the Sylvian fissure, corresponding to the SII cortex. Furthermore, in order to compare the ECD locations, we normalized their x-, y-, and z-coordinate values by defining those of theta activities elicited by moderate pain as 0, 0, and 0, respectively. Figure 4(B) shows the relative values (mean ± s.d) and distributions across the ten subjects of the theta and alpha activities for each stimulus condition. No significant difference for the factor of pain intensity was identified ($\chi^2 < 2$, $p > 0.1$).

**DISCUSSION**

In this study we used wavelet analysis and equivalent current dipole modeling to analyze the temporal and spatial characteristics of neuromagnetic oscillatory activities in bilateral SII areas following painful laser stimulation at varying pain intensities. Our first finding was the significant power increase...
of delta to alpha frequency band activities in the bilateral SII areas 180 to 210 ms following noxious stimulation. This result is partly in line with previous EEG studies using median nerve stimulation, intracutaneous electrical stimulation and CO2 laser skin stimulation. It is also in accordance with the ideas of neuronal processing with simultaneous oscillations in various frequency bands. Remarkably, the salient theta and alpha activities were observed in all pain intensity conditions in the bilateral SII areas. In contrast with our findings, however, some EEG studies showed a decrease in alpha and an increase in beta oscillations following tonic thermal or chemical stimulations. Babiloni and coworkers reported an increase of theta to gamma activities in the contralateral hemisphere and a decrease of beta activity in the ipsilateral hemisphere following painful electrical stimuli. This divergence might be related to discordant pain-eliciting modalities and different analysis methodologies. It is noteworthy that our study has its strengths in the selective activation of nociceptive neurons and precise localization in time, space and frequency, in comparison with previous EEG spectral analyses which used Fourier transform. We therefore believe that the theta and alpha activities are essentially engaged in cortical pain processing. The functional significance of the cortical oscillations in the SII cortex has, however, remained somewhat unclear. One recent MEG study...
has reported 7-9 Hz rhythm in the human SII cortex, probably processing the bilateral tactile inputs. We therefore propose that theta and alpha activities in the SII areas may play an important role in processing nociceptive inputs.

In this study, the latency range of the bilateral oscillatory activities is in agreement with that reported in earlier LEP, and LEF studies. Moreover, the latency between 180 and 210 ms, irrespective of the pain intensity and the hemispheres, may suggest that the sensory input is conducted via the ascending Aδ fibers. No hemispheric lateralization of nociceptive processing in SII areas was found to result from there being no significant power difference in oscillatory activities between the contralateral and ipsilateral hemispheres in all pain intensity conditions. Coghill and colleagues also suggested that pain intensity-dependent activation of the SII cortex was predominantly bilateral in their positron emission tomography (PET) studies. Moreover, psychophysical studies of patients with one cerebral hemisphere excision or split-brain have also confirmed that both cerebral hemispheres can independently process pain intensity information. Our present observation of similar oscillatory activities between the bilateral SII areas supports this idea of the bilateral mechanism being engaged in the nociceptive processing.

In this study, the power increase of theta and alpha activities, when correlated with pain intensity levels, showed a tendency to increase from a moderate to a severe condition, although this tendency was not significant. Recent studies on EEG responses to CO₂ laser skin stimulation have suggested that event-related synchronization activities from delta to alpha bands, with a mean latency of 225 ms, increase in positive correlation with stimulus strength from 3-11 mJ/mm². Mouraux et al have reported a change of event-related synchronization from non-painful to mild painful stimulation. In our study, however, a dissimilar stimulus paradigm was used as the mean stimulus strengths were calculated to be 25.5, 36.5 and 49 mJ/mm² for mild, moderate and severe pain, respectively. We found that the theta and alpha activities in the SII areas code for stimulus strengths of up to 36.5 mJ/mm² or up to a moderate pain intensity. The difference in energy densities used by Mouraux et al and the present study may be explained by the difference in wavelength of the laser stimulators used. As the skin has a higher transparency for the shorter wavelength of the thulium-YAG laser, as compared to the CO₂ laser, much more caloric energy is needed to bring a larger volume of tissue above the activation threshold of nociceptors. Raij et al have shown that the amplitude of LEF responses increases strongly along with the increment of interstimulus interval (ISI) from 0.5 s to 4 s, and then reaches a plateau at the ISI of 8 to 16 s. Moreover, unlike CO₂ laser stimuli, our thulium-laser stimuli last for only 1 ms. It seems not possible for an acclimation to the stimuli to be developed during our experiments with ISI of 8-12 s. Moreover, in our present study, the order of various experimental sessions (mild, moderate, and severe pain) was randomized, and the subject had a five-ten minute rest before each session. Thus, the lack of significant change between moderate and severe pain conditions cannot be ascribed to the acclimation effect.

In both hemispheres, the spatial distributions of theta and alpha activities to nociceptive information processing closely resembled each other in all pain-intensity conditions. The oscillations were centered in the SII region in the bilateral hemispheres, in accordance with previous scalp EEG, intracranial EEG, and MEG recordings. We therefore suggest that there is no spatial separation of the generators for the rhythmic activities elicited by varied pain intensity within the SII areas.

In conclusion, power increases of 4-13 Hz oscillations peaking from 180 to 210 ms play an important role in processing Aδ nociceptive inputs in the bilateral SII areas. Theta and alpha activities in the SII areas reflect the perceived pain magnitude up to a moderate degree, rather than a full scale, of pain rating. These rhythmic generators elicited by different pain intensity heavily overlapped within the SII areas.

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