Neurophysiological evaluation of convergent afferents innervating the human esophagus and area of referred pain on the anterior chest wall

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Visceral nociceptors terminate predominantly in lamina I and V of the spinal dorsal horn and are thought to converge here with both unmyelinated (lamina I) and thin myelinated (lamina I and V) somatic afferents (32). These afferent inputs ascend via shared signaling pathways and are processed in many common cortical and subcortical regions (14, 18). The advent of human functional brain imaging studies has provided confirmatory evidence that many brain regions process both visceral and somatic pain, with the major differences appearing to be the diffuse activation of bilateral brain regions during visceral pain compared with somatic (3, 8, 9). It is of interest to note, however, that direct stimulation of the secondary somatosensory cortex/posterior insula in humans can evoke strong sensations of somatic pain whereas visceral pain has only been elicited following stimulation of the anterior cingulate cortex and amygdala (22, 25, 26), suggesting subtle differences in cortical organization may exist.

Another unique property of visceral pain is characterized by its referral to somatic regions that are innervated from the same spinal segments (13). The precise mechanism of referred pain has yet to be unequivocally determined, although several complementary theories exist (23, 29). We have previously shown that after sensitization of the esophagus with experimental acidification, central sensitization of spinal dorsal horn neurons occurs and large myelinated Aβ fibers that innervate the area of referred pain on the anterior chest wall become hypersensitive. These fibers normally only process innocuous tactile stimuli but once sensitized become capable of signaling pain, resulting in allodynia (34).

Sensitization of the viscera may also induce hypersensitivity within somatic pain fibers that comprise thinly myelinated Aδ and unmyelinated C fibers within the region of referred pain. This has yet to be unequivocally demonstrated in humans because electrical stimulation, which is commonly used to objectively evaluate the neurophysiological characteristics of afferent pathways, coactivates Aβ, Aδ, and C fibers when applied to the skin, producing a mixed fiber response.

Recently, a contact heat thermode that rapidly and selectively activates somatic nociceptors has been developed commercially (2, 28). This device allows selective comparison of nociceptive afferent function from somatic regions of pain referral with their corresponding visceral pain-initiating site. In addition, the rapid onset of the thermal stimulus allows discrete afferent volleys from predominantly Aδ fiber afferents to be evoked which are extremely well suited for cortical evoked

THE DISTINCT PSYCHOPHYSICAL properties of somatic and visceral pain reflect the biological and survival imperatives required by the host organism to respond appropriately and efficiently to different noxious events (20, 35). Most of the gastrointestinal tract has sparse nociceptive innervation, but these afferents diverge as they exit the viscera, entering the spinal cord via the dorsal root ganglia across multiple spinal segments (11, 12, 18).
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potential (CEP) recordings. The ability to noninvasively and objectively investigate viscerosomatic sensitization using this method would be of great benefit in understanding better the mechanisms of referred pain and for evaluating patients that present with referred pain symptoms.

The aim of this study was twofold: first, to compare the cortical processing and conduction characteristics of nociceptive afferent inputs from the esophagus and area of referred pain on the anterior chest wall; second, to determine the test-retest variability of this technique and examine selected psychophysical measures may contribute to the inherent physiological variability encountered within the study.

MATERIALS AND METHODS

After ethical approval was gained from the local ethics committee, 12 healthy volunteers (10 male, 2 female; mean age 40 ± 7.9 yr), free from any neurological, metabolic, or inflammatory condition, were recruited from the GSK Clinical Unit Cambridge volunteer panel. Volunteers attended the unit for screening, 3 study days, and a follow-up visit. Screening occurred within 28 days of the first study day.

All healthy subjects underwent esophageal manometry which had to be normal for inclusion into the study, and these data were used to determine the location of the lower esophageal sphincter to aid positioning of the esophageal stimulation catheter. On each study day assessments included esophageal sensory testing by electrical stimulation, somatic pain assessment of the area of referred esophageal pain by thermal stimulation, and CEP recordings from both of these regions.

Esophageal sensory testing. The esophageal stimulation catheter housed a pair of bipolar, platinum ring electrodes (2-mm electrodes with an interelectrode distance of 1 cm). The bipolar ring electrodes were connected to a constant-current, high-voltage stimulator (model D57, Digitimer, Welwyn Garden City, UK) and externally triggered (model DSN, Digitimer). The interelectrode impedance was monitored throughout to ensure mucosal contact, and the epoch was rejected if mucosal contact was shown, as assessed via an impedance monitor, to be insufficient (>10 kΩ). The stimuli were 500-μs duration square-wave pulses, and we used a maximum intensity of 100 mA. These stimulation parameters have been used in several previous studies and do not produce any adverse cardiac events (30).

Chest wall heat pain and tolerance testing. Heat pain threshold and tolerance were assessed on the area of referred pain generated by the electrical stimulation of the esophagus. Once identified, this site on the anterior chest wall was tested by use of the Medoc (3 cm × 3 cm) TSA II NeuroSensory Analyzer (Medoc, Ramat Yishai, Israel). Heat pain threshold was recorded as the temperature at which the first sensation of pain was felt via the ascending method of limits. Heat pain tolerance was recorded as the maximum temperature that the subjects could tolerate. During the testing period, the temperature of the thermode was gradually increased from the baseline (32°C) at a rate of 1°C/s. TSA has an automatic cutoff limit to prevent burn injury.

CEP. CEP were recorded from 12 standard EEG Ag-AgCl disk electrodes along the midline of the scalp at electrode positions Fz, FCz, Cz, CPz, Pz, POz (extended 10–20 electrode placements). Reference electrodes were placed at the mastoid process (M1/M2) or earlobes (A1/A2) bilaterally, and two ground electrodes were applied, one cephalic and one noncepalic. Two electrodes were also placed above and below the right eye, in line with the pupil (in neutral gaze) to allow ocular artifact correction. Electroencephalographic potentials were recorded and averaged during stimulation the esophagus (visceral) and chest wall (somatic) sites by using parameters outlined below.

The evoked potential data were recorded on a Neuroscan System (Advance Medical Equipment, Horsham, UK) with a sampling rate of 1,000 Hz and effective voltage resolution of 0.084 μV/bit. The filter was set at 100 Hz low pass and 0.15 Hz high pass, and impedance was kept below 5 kΩ. The epoch lengths were set at 200 ms prestimulus and 2,600 ms poststimulus.

CHEPS. The thermal stimulation for the chest wall evoked potentials requires a rapid onset stimulus, which was provided by the Medoc contact heat evoked potential stimulator (CHEPS) system (Pathway model, Medoc). CHEPS delivers fast repetitive heat stimuli of predetermined temperatures resulting in a selective activation of somatic nociceptors to allow CEP recording. The CHEPS thermode was ~27 mm in diameter and was placed in direct contact with the skin on the anterior chest wall. The heating rate was 70°C/s and the maximum temperature used was 50°C.

Psychological questionnaires. The Fear of Pain Questionnaire-III (FPQ) was administered at the beginning of the study on visit 1. This questionnaire comprises 30 questions and measures fears about pain (21). The Bond and Lader mood scale (4) was used at the beginning of the study to assess anxiety, dysphoria, and sedation on each visit. During the study (postintubation) an anxiety visual analog scale (VAS) was used to determine state anxiety levels also on each of the three visits.

EXPERIMENTAL PROCEDURES

Esophagus. At the beginning of each study, the esophageal stimulation catheter was passed either orally or nasally so that the bipolar ring electrodes were sited at 5 cm above the lower esophageal sphincter. Initial measurements of sensory perception and pain thresholds were determined by increasing the electrical stimulus intensity in 2-mA increments until the subjective end points were reached as indicated verbally by each subject. This procedure was repeated three times and average values were obtained. In addition, 75% pain threshold was calculated as the stimulation intensity to be used for esophageal evoked potentials (EEP) as previously reported (15).

EEP were recorded by averaging a total of 200 esophageal stimuli, acquired at a frequency of 0.2 Hz, during four runs of 50 stimuli, with a 10-min interrun interval. The parameters used were selected on the basis of our previous work to provide optimal EEP responses (15). After each run of 50 stimuli, the subjects were asked to score the esophageal sensation experienced using a six-point categorical rating scale: 1, Unaware; 2, Slight sensation; 3, Definite sensation; 4, Slight Discomfort; 5, Uncomfortable; and 6, Painful. In previous studies, we have shown a direct relationship between the sensory perception score and the values for amplitude and latency of EEP components, with the amplitude increasing and latency decreasing with increasing perception of the stimulus (17).

Anterior chest wall. After the esophageal pain threshold was determined, subjects were required to identify the area on the anterior chest wall to which esophageal pain generated by the intraluminal electrical stimulus was referred. This area was marked and used for somatic heat pain assessments. If this area was deemed to be covered too thickly with chest hair, the area was gently clipped to improve thermode contact. Subjects were positioned in a semirecumbent position and asked to relax during the stimulation paradigm. The heat ramp stimulus began at 32°C and rose in temperature at 1°C/s to a maximum of 50°C. The ramp was stopped by the subject pushing a button on a response unit when the heat pain threshold was reached and the temperature of the thermode returned to baseline. This was repeated three times, and an average value of the heat pain threshold temperature was recorded. This was repeated for maximum heat pain tolerance. Once these values had been determined, CEP were recorded at baseline by averaging 40 stimuli, at a frequency of 0.125–0.083 Hz, delivered in two runs of 20 stimuli with a 5- to 10-min interrun interval by using a stimulus intensity of 50°C in all subjects. The rise time of the thermal stimulus from baseline to 50°C was ~250 ms.

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reduce the effects of peripheral receptor habituation the thermode was hand held during the stimulation procedure and moved slightly, within the area of somatic referral, in between each stimulus presentation. The volunteers were asked to report their perceived pain for each stimulus 2–3 s after each stimulus was delivered by use of an 11-point numerical rating scale (0 = no pain, 10 = worst pain imaginable). These parameters had been determined in house as producing high-quality CEP responses (2, 28).

Data analysis. All values are reported as means along with 95% confidence intervals. The latency of CEP components were initially measured from the onset of the stimulus to the maximal peak of each component and reported in milliseconds (ms). The CEP amplitudes were measured as the maximum peak-to-peak difference between consecutive components and reported in microvolts (μV). The first positive (downward) component of the CEP waveform is labeled as P1 with the first negative (upward) component labeled N1. The second positive component is labeled P2. Values for electrical stimulation intensities are given in milliamperes (mA) and for heat in degrees centigrade (°C). Data are displayed as means ± ISD. Statistical analysis consisted of an ANOVA, with values reported as point estimates of difference ± 95% confidence interval, and Spearman rank correlation (r = rho). Visit 1 (V1), visit 2 (V2), and visit 3 (V3) represent the 3 experimental study days.

RESULTS

Eleven subjects completed the study, with one subject missing visit 3 only because of illness. No procedure-related adverse events were reported.

Esophageal pain and sensory perception thresholds. Esophageal sensory perception thresholds increased over time [V1 = 23.94 mA ± 7.4, V2 = 23.92 mA ± 5.1, V3 = 28.91 mA ± 4.45, V1-V3 difference = −4.91 mA (−9.96, −0.15) P = 0.056]. Esophageal pain thresholds increased over time [V1 = 49.8 ± 16, V2 = 56.1 ± 16, V3 = 67.7 ± 19, V1-V3 difference = −17.9 mA (−27.9, −7.9) P < 0.001].

Chest wall pain threshold and tolerance. Chest wall pain thresholds increased over time [V1 = 45.1°C ± 4.5, V2 = 48.3°C ± 2.4, V3 = 48.8°C ± 1, V1-V3 difference = −3.38°C (−5.33, −1.42) P = 0.001]. Chest pain tolerance also increased over time [V1 = 48.02°C ± 2.47, V2 = 48.98°C ± 0.78, V3 = 49.17°C ± 0.86, V1-V3 difference = −0.91°C (−1.77, −0.06) P = 0.03].

EEP. All values for EEP are given in Table 1. Although there were no significant differences between the P1 latency of the EEP component over the three visits, the N1 and P2 peak latency decreased on V3. There were no changes in the amplitude of EEP components over the three visits. Figure 1 shows a typical example of EEP in one subject over the three visits.

ACWEP. All values for anterior chest wall heat pain evoked potentials (ACWEP) are given in Table 2. There were no significant differences between the latency of the P1 and N1 ACWEP components over the three visits. The P2 component increased in latency when V1 values were compared with V3. In addition, the amplitude of the P1-N1 and P2-N2 components decreased significantly over time, reflecting habituation to the 50°C stimulus over time. Figure 2 shows a typical example of ACWEP in one subject over the three visits.

Comparison of visceral and somatic evoked potential latencies. Once the 250-ms rise time of the thermal probe stimulation is taken into account and subtracted from the mean latencies of the ACWEP components, the average P1 latency was 101.6 ms compared with 126.6 ms for the P1 latency of the EEP. These latencies are comparable to those previously reported for EEP and approximate to a conduction velocity of 8–10 meters/s, indicating afferent transmission via thinly myelinated Aδ fiber afferents (17).

Psychological assessments. There was a significant negative correlation between the FPQ scores and esophageal pain threshold on V1 (r = −0.57, P = 0.05), which was the visit when subjects completed the FPQ (Fig. 3). There was no correlation of FPQ with somatic heat pain thresholds (r = 0.01, P = 0.97). The postintubation anxiety VAS scores reduced significantly over time [V1 = 21.1 mm ± 19, V2 = 14.6 mm ± 14, V3 = 10 mm ± 10.4, difference V1-V3; 7.88 (2.34, 12.3), P = 0.001].

Table 1. Latency and amplitude of esophageal evoked potential components

<table>
<thead>
<tr>
<th></th>
<th>V1 (ms)</th>
<th>V2 (ms)</th>
<th>V3 (ms)</th>
<th>V1–V3 Comparison</th>
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<tbody>
<tr>
<td>P1, ms</td>
<td>119.6±29</td>
<td>132.6±14</td>
<td>129.4±19</td>
<td>−5.43 (−18.1, 7.2) P = 0.382</td>
</tr>
<tr>
<td>N1, ms</td>
<td>184.2±34</td>
<td>183.4±23</td>
<td>178.1±21</td>
<td>13.7 (1.8, 25.5) P = 0.026</td>
</tr>
<tr>
<td>P2, ms</td>
<td>300.4±43.9</td>
<td>295±38</td>
<td>272.4±23</td>
<td>32.5 (11.8, 53.3) P = 0.004</td>
</tr>
<tr>
<td>P1-N1, μV</td>
<td>2.05±1.6</td>
<td>2.48±1.8</td>
<td>1.95±1.9</td>
<td>0.04 (−1.3, 1.3) P = 0.950</td>
</tr>
<tr>
<td>N1-P2, μV</td>
<td>1.4±2.2</td>
<td>1.6±2.3</td>
<td>2.19±2.5</td>
<td>0.85 (−0.2, 1.9) P = 0.103</td>
</tr>
<tr>
<td>P2-N2, μV</td>
<td>8.2±5.2</td>
<td>6.5±4.5</td>
<td>7.3±3.4</td>
<td>−0.4 (−2.2, 1.4) P = 0.628</td>
</tr>
</tbody>
</table>

Values are group means ± 1 SD. Latency is measured in milliseconds and amplitude in microvolts. Statistical comparisons are reported as point estimates of difference [95% confidence interval (95% CI)]. V1–V3, visits 1–3.
decreased sensitivity reported by subjects over time. In patients pre- and postcholecystectomy, the authors showed that prior to surgery, 84% of patients had somatosensory hyperalgesia in the area of referred pain which resolved within 4–12 wk following surgery (31).

Many of the studies have relied on quantitative sensory testing and subjective reporting of evoked sensations, which can be liable to response bias and do not allow for objective assessment of the different neural pathways involved in mediating the response (33). We have previously shown that esophageal acidification can induce somatic hypersensitivity and allodynia to electrical stimulation of the area or referred esophageal pain on the anterior chest wall (30, 34). This somatic allodynia is thought to develop following esophageal tissue injury-induced central sensitization of spinal cord neurons. As the receptive fields of afferent neurons from the affected area expand, they encompass regions of referral with mixed results (23). More recently, referred visceral pain has been suggested as a biomarker of central sensitization in studies of patients with various chronic visceral pain conditions.

Drewes and colleagues (5–7, 31) have performed many studies looking at referred visceral pain both in health and in patients with conditions that include gastroesophageal reflux disease (GERD), noncardiac chest pain (NCCP), pancreatitis, and gallstone disease. In studies of healthy subjects it has been shown that sensitization of the esophagus with either dilute acid or capsaicin results in the expansion of the area of referred esophageal pain elicited by thermal, mechanical, and electrical stimulation (1, 24). In addition, patients with GERD and NCCP also demonstrate increased areas of referred esophageal pain compared with healthy controls (6, 7). In a well-designed study in patients pre- and postcholecystectomy, the authors showed that prior to surgery, 84% of patients had somatosenory hyperalgesia in the area of referred pain which resolved within 4–12 wk following surgery (31).

Fig. 3. Scatterplot depicting the correlation between Fear of Pain Questionnaire 3 scores and esophageal pain thresholds.
the spinal cord that receive afferent input from large myelinated A\(\beta\) fibers, which normally only process innocuous stimuli. This results in A\(\beta\) fibers signaling to the sensitized region of the dorsal horn and innocuous stimuli being perceived as pain (37). We provided objective neurophysiological evidence to support this by recording somatosensory evoked potentials from the area of referred esophageal pain pre and postacid and showed that the amplitude of the somatosensory evoked potential increased and the latency decreased, demonstrating amplification of the large-diameter myelinated (A\(\beta\)) fiber signaling pathway that is selectively activated at very low levels of electrical stimulation (34).

Although somatic allodynia is predominantly mediated via A\(\beta\) fibers, A\(\delta\) and C fiber nociceptors also innervate cutaneous structures. A\(\delta\) fibers are thinly myelinated and are responsible for encoding “first” pain sensations, which are generally quite well localized and sharp in nature. C fiber nociceptors mediate “second” pain sensations and are characteristically poorly localized and burning in nature (27). It is likely that both types of nociceptors contribute to the sensations of referred visceral pain, but it has not been possible to objectively dissect out their contribution with previous techniques.

Recent advances in technology have led to a new generation of thermal stimulators, which selectively activate A\(\delta\) and C fiber nociceptors but not A\(\beta\) fibers. In addition, the rapid onset of the thermal stimulation has made it possible to record CEP in response to repeated stimuli. This has meant that the neurophysiological characteristics of the nociceptive signaling pathway from somatic structures can be studied in greater detail (28). Previous studies have validated this methodology, and it has begun to provide valuable insights into the pathophysiology of several neuropathic pain conditions (2).

In this study we demonstrated that CEP recorded following esophageal and anterior chest wall stimulation are identical in morphology consisting of a P1-N1-P2-N2 complex, thus suggesting that pain processing of these two regions occurs within a similar cortical network. In addition, the peak latencies of the visceral and somatic CEP were also similar, indicating mediation via thinly myelinated A\(\delta\) fibers. Our ability to objectively assess these pain signaling pathways offers several important advances.

By not relying on subjective reports, we can reduce some of the inherent variability encountered in most sensory testing studies. This is important because psychological factors can often play as big a role in reporting hypersensitivity, as can peripheral injury. This has been demonstrated in a study of NCCP patients and in patients that present with neuropathic pain-like symptoms (10, 16). Both of these studies have shown that despite patients presenting with almost identical clinical profiles, they can be subgrouped on the basis of the neurophysiological characteristics of the CEP response into those with an overt peripheral sensitization vs. those that are “hypervigilant” and overreport symptoms because of predominantly top-down processes often driven by stress or other psychological factors (10, 16). Indeed, even in this healthy volunteer study, we saw that subjects’ anxiety levels decreased over time as they acclimatized to the study procedures, and this was accompanied by an increase in pain thresholds. In addition, there was a significant negative correlation between subject’s FPQ scores and esophageal pain threshold. Since visceral pain can be perceived as more threatening than somatic pain, this is an interesting finding and again points to the important role of psychological factors in understanding the range of sensitivity encountered within a study population.

The protocols for esophageal and chest evoked potentials differed slightly because the esophageal stimulation intensity used to evoke the cortical responses was always calculated as a percentage of the subject’s pain threshold on that particular day (15). As pain thresholds increased between visits, then the stimulation intensity used also increased, and this was reflected by a reduction in the latency of some evoked potential components, which is commonly seen when the stimulation intensity is increased. Conversely, the thermal stimulation used the same stimulation intensity over the three visits but somatic pain thresholds also increased over this time. This was reflected by the decrease of chest evoked potential amplitudes and an increase in the P2 latency. This not only shows the sensitivity of evoked potentials to reflect subtle changes in sensitivity over time but also highlights the importance of factoring in habituation into experimental protocols that use repeated measures to determine outcomes. The fact that habituation to pain is often absent or reduced in patients with functional pain syndromes makes this an important observation to consider in future studies (19, 36).

Even though variability in evoked potential values was seen across the study days, this was easily explainable by physiological phenomena and therefore the methodology used appears to be remarkably robust. As long as the factors we have discussed are taken into account, the evoked potential responses were very consistent across the study visits and the procedure was well tolerated.

In summary, we have shown that esophageal and referred esophageal pain share common signaling pathways and are processed within a similar cortical network. This study provides the foundation to use this methodology to further understand mechanisms of referred pain in humans and may provide a noninvasive clinical investigation tool for the assessment of viscerosomatic sensitization in patients with a variety of clinical conditions.

DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

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