Distinct neurophysiological profiles in irritable bowel syndrome

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ABSTRACT

The subjective end point of pain or discomfort when assessing visceral sensation remains inadequate because of susceptibility to response bias related to the psychological state, with a higher tendency of patients with functional gut disorders to report pain (4, 31). The lack of a single robust objective clinical marker to quantify visceral hypersensitivity has hindered progress in IBS drug development. Much of this difficulty lies in the complex multifaceted nature of the condition, compounded by the strong emotional and neurobehavioral components of pain.

Surrogate measures of visceral pain can be obtained from visceral function studies, such as the barostat, or metabolic neuroimaging techniques, such as PET and functional MRI (1, 18). Evaluation of pain using these tools had several limitations, including anticipation, habituation, and lack of non-stimulus-specific activity (23, 33). Some of the limitations were overcome by magnetoencephalography (MEG) (15). However, as MEG is available in only a few centers worldwide, extrapolation of MEG data to EEG recording of cerebral evoked potentials (CEPs) was subsequently developed as a possible clinical tool with an objective evaluation of visceral pain. CEPs are measurements of electrical potentials, generated by cortical neurons following a series of repeated sensory stimuli, recorded using scalp surface electrodes. Stimulus-specific CEPs occur at a fixed time after each stimulus, while brain-gut interaction; visceral sensitivity; gastrointestinal physiology

ABDOMINAL PAIN IS THE CARDINAL symptom of irritable bowel syndrome (IBS), with associated diagnostic symptoms such as alterations in stool frequency or consistency (7). Visceral hypersensitivity is a recognized physiological process associated with pain in IBS (20). Several mechanisms may contribute to the low pain threshold observed in this condition. Peripheral sensitization of visceral afferent nerves activated by previous injury or infection, mediated via inflammatory mediators such as prostaglandins, is closely integrated with central sensitization of spinal afferents (21, 26). Once chronic sensitization is established, the two processes become indistinguishable, as they contribute to the development of visceral hypersensitivity in functional gut disorders (5, 21, 25, 26). More recently, perception of gut stimuli and postprandial symptoms in IBS were shown to be enhanced during stressful conditions (10, 22). As the treatment and management strategies deployed to ameliorate these different aberrant mechanisms are distinct (i.e., analgesics vs. psychological therapy), identifying the predominant underlying pathophysiology remains an important clinical objective.

The objective of this study was to determine whether cortical evoked potentials (CEPs) can define neurophysiological patterns in irritable bowel syndrome (IBS). In this prospective study of consecutive patients attending secondary and tertiary centers, patients with Rome II-defined IBS underwent rectal sensory and pain threshold (RST and RPT, respectively) testing with electrical stimulation on three separate visits. CEPs were collated for 75% pain thresholds, and anxiety [Spielberger State-Trait Anxiety Inventory (SSTAI)] questionnaires were completed. Subjects were 33 IBS patients (27 female, mean age 40.1 yr) and 21 healthy controls (14 female, mean age 31.4 yr). At visit 3, RPT was significantly lower [mean (95% CI)] in IBS patients than in control subjects: 58.2 mA (48.0–68.5) vs. 79.5 mA (69.3–89.6) (P < 0.01). No significant differences were observed in CEP latencies and amplitudes between visits 1, 2, and 3 within each group, except P2 latency for controls (P = 0.04) and N2 latency (P = 0.04) and N2 amplitude (P = 0.02) for IBS patients. Group comparisons showed significant differences in 3-day mean RPT, CEP amplitudes, and CEP latencies between IBS patients and controls. RPT <50 mA and P1 latency >106 ms were identified for four IBS subgroups: 24% were hypersensitive, 12% were hypervigilant, 15% were hyposensitive, and 49% exhibited normal P1 latency and pain threshold. CEPs are reliable and reproducible measures of early sensory processing. Identification of four IBS neurophysiological patterns highlights its heterogeneous nature. These findings mark the first step toward personalized medicine in IBS, whereby therapy may be directed at the underlying physiological process.

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The purpose of the present study was to determine whether distinct neurophysiological phenotypes occur in IBS. The primary aim was to test the reliability of CEPs in response to rectal electrical stimulation over time in IBS. Secondary aims were to correlate these profiles with sensory and psychological measures.

MATERIALS AND METHODS

Consecutive IBS patients were recruited from the outpatient clinics at a tertiary and a secondary care hospital. Patients were included if they fulfilled the Rome II criteria for IBS. Investigations were undertaken at the discretion of the attending physician. Exclusion criteria included unexplained weight loss, psychiatric disorder, acute infection, recent antibiotic treatment, antidepressant therapy, anxiolytics, and anti-inflammatory or pain medication. Healthy volunteers were recruited consecutively as the control group by local advertisement. All participants gave informed consent. The study was approved by the Harrow Ethics Committee.

Protocol

Each participant underwent 3 nonconsecutive days of neurophysiological assessment within a 3-mo period. At each visit, subjects underwent rectal electrical stimulation to determine sensory and pain thresholds; CEPs to the rectal stimulus were subsequently recorded. Female patients were tested during the follicular phase of the menstrual cycle.

Assessment of Rectal Sensitivity

Rectal stimulation. The rectal catheter (Gaeltec, Dunvegan, Isle of Skye, Scotland, UK) featured two 2-mm bipolar ring electrodes sited 1 cm apart. The electrodes were positioned 10 cm proximal to the anal verge. The catheter was connected to a constant-current stimulator (model DS7A, Digitimer), which was controlled by a trigger generator (model DG2, Digitimer). The stimulus frequency was 0.5 Hz using 500-µs square-wave pulses. The intensity of the stimulus was manually adjusted between 0 and 100 mA.

Visual analog scale. Subjects graded the stimulus intensity using a visual analog scale (VAS) scored between 1 and 6 as follows: “unaware of the stimulus” was scored as 1, “slight sensation” as 2, “definite sensation” as 3, “slight discomfort” as 4, “uncomfortable” as 5, and “painful” as 6. This scale has been used previously in CEP studies and showed correlation between stimulation intensity and CEP responses (16).

Rectal sensory and pain thresholds. Rectal sensory and pain thresholds (RST and RPT, respectively) were determined by assessing response in 2-mA increments. The first electrical stimulus perception was defined as the sensory threshold, or VAS = 2. Further stimulus intensity increases were recorded via VAS until sensation was perceived as painful (defined as the pain threshold, or VAS = 6). This ascending electrical stimulation cycle was repeated twice to calculate average RST and RPT over three cycles. The stimulus intensity used to elicit CEP was equivalent to the RST plus 75% of the difference between the RST and RPT (75% RPT) and has been validated in previous studies (13).

Assessment of CEPs

Placement of cortical electrodes. Silver-silver chloride surface electrodes were placed on the vertex (active electrode), earlobe (reference electrode), and neck (ground electrode) according to the International 10-20 System of EEG electrode placement. The electrodes were fixed using electrode gel or paste (Elebix, Nihon Kohden) after the skin was scrubbed with a mild abrasive (Omniprep, Weaver & Aurora) to maintain scalp impedance at <5 kΩ.

CEP recordings. CEPs were recorded with subjects lying still and their gaze fixed on a stationary object to minimize motor artifacts. The rectal stimulus was delivered at the calculated testing intensity over four cycles of 50 electrical stimulations spaced at 5-s intervals. A 5-min pause interspersed each cycle. Each cycle of 50 stimulations was averaged to enhance signal clarity. The patient was given 5 min to relax after each cycle before testing resumed. At the conclusion of each day of testing, four averaged CEP waveforms were calculated.

CEP acquisition and analysis. Data were acquired using a programmable signal conditioner (model 1902, Cambridge Electronics Design), as previously described (13). Rectal CEPs were analyzed according to morphology, latency, and amplitude of the waveforms. CEPs were averaged after each cycle and then compared with other cycles on the same day of testing (intraday analysis) and on other days of testing (interday analysis). Four main CEP components were recognized in previous studies.

Definition of terms. The main CEP peak components are P1, N1, P2, and N2. Neurophysiological convention describes negative potentials as upward deflections (N1 and N2) and positive potentials as downward deflections (P1 and P2). Latency refers to time (in ms) from the stimulus trigger to the peak component. Amplitude is defined as the voltage difference between consecutive CEP peaks (Fig. 1).

Early recorded CEP complex represents stimulus-specific activation of the primary or secondary somatosensory cortex and insula and depends on transmitted afferent signal strength intensity. Cortical activity of ~250 ms or more after stimulation reflects brain endogenous processing of theafferent information and represents a more general “arousal”-type response. The relationship between CEP morphology and pain thresholds was examined in a previous study (16) and defined as follows. 1) Afferent hypersensitivity refers to pain perception at relatively low stimulus intensity with a normal-latency P1 component. 2) Hypervigilance refers to pain perception at low stimulus intensity, but with prolonged-latency P1 responses (indicating that afferent signaling pathways are not sensitized, instead patients were “amplifying” normal-intensity stimuli once the stimulus reached the brain). 3) Hyposensitivity refers to maximum stimulus application (limited by the stimulator and safety reasons) without eliciting a normal CEP response.

Withdrawals. All participants completed the first day of CEP testing (visit 1). At visit 2, two IBS patients and two controls dropped out for the following reasons: failure to attend, technical failure of electronic CEP data capture, pregnancy, and difficulty in attending hospital. At visit 3, a further two IBS patients withdrew: one failed to attend, and the other underwent unrelated surgery.

Reference values. The results from the healthy controls were used to define reference values for normal pain threshold and P1 latency.

Fig. 1. Diagrammatic representation of rectal cortical evoked potential (CEP) components. Diagram shows positive and negative deflections following a stimulus. Neurophysiological convention describes negative potentials as upward deflections (N1 and N2) and positive potentials as downward deflections (P1 and P2). Latency refers to time (in ms) from stimulus trigger to peak component. Amplitude is defined as voltage difference between consecutive CEP peaks. Markers for P1 latency (P1L) and P1 amplitude (P1A) are shown.
Table 1. Demographics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 21)</th>
<th>IBS Patients (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr [mean (range)]</td>
<td>31.4 (21–46)</td>
<td>40.1 (20–63)</td>
</tr>
<tr>
<td>Sex, M/F [n (%)]</td>
<td>14/7 (66.7)</td>
<td>18/15 (54.5)</td>
</tr>
<tr>
<td>Type of IBS, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Alternating</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Unclassified</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Mean ages and sex distribution were similar in each group. Most irritable bowel syndrome (IBS) patients had diarrhea-predominant IBS.

Psychological Profile

Differences in anxiety between study groups faced with the same unknown testing situation were examined using the Spielberger State-Trait Anxiety Inventory (STAI), which measures state (STAI-state) and trait (STAI-trait) anxiety and overall anxiety levels. Scores range from 1 to 20, with higher scores indicating greater anxiety (29). Each questionnaire has 20 questions, with 4 possible Likert-type responses scored between 1 and 4. Scores >35 in either test were considered to be pathological.

Data Collection and Statistics

The RST and RPT (mA) are presented as means with 95% confidence intervals [means (95% CI)]. CEPs demonstrated characteristic morphology with P1, P2, P3, N1, and N2 waves were measured for 75% RPT. Data are presented as mean differences between visits 1, 2, and 3, with 95% CI to highlight the reproducibility of the measurements. Absolute values are also expressed as means (95% CI). As this was an exploratory study, we decided on sample number by considerations. Absolute values are also expressed as means (95% CI). As this was an exploratory study, we decided on sample number by considerations. Absolute values are also expressed as means (95% CI). As this was an exploratory study, we decided on sample number by considerations. Absolute values are also expressed as means (95% CI). As this was an exploratory study, we decided on sample number by considerations. Absolute values are also expressed as means (95% CI). As this was an exploratory study, we decided on sample number by considerations. Absolute values are also expressed as means (95% CI). As this was an exploratory study, we decided on sample number by considerations. Absolute values are also expressed as means (95% CI).

RESULTS

Demographics

Thirty-three Rome II IBS patients (27 female, mean age 40 yr) and 21 healthy controls (14 female, mean age 31 yr) were recruited. Demographic data are shown in Table 1. There were no significant differences in age and sex between the two groups.

Table 2. Sensory and pain thresholds in controls and IBS patients

<table>
<thead>
<tr>
<th></th>
<th>Visit 2 vs. Visit 1</th>
<th>Visit 3 vs. Visit 1</th>
<th>Visit 3 vs. Visit 2</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RST</td>
<td>-3.3 (-7.2 to 0.5)</td>
<td>-2.2 (-6.2 to 1.7)</td>
<td>1.1 (-2.9 to 5.1)</td>
</tr>
<tr>
<td>RPT</td>
<td>1.4 (-12.1 to 4.9)</td>
<td>9.2 (-4.4 to 22.9)</td>
<td>7.8 (-6.0 to 21.7)</td>
</tr>
<tr>
<td>75% RPT</td>
<td>0.13 (-10.8 to 11.1)</td>
<td>6.2 (-4.9 to 17.3)</td>
<td>6.1 (-5.2 to 17.3)</td>
</tr>
<tr>
<td>IBS patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RST</td>
<td>2.6 (-3.1 to 8.3)</td>
<td>-2.5 (-8.2 to 3.3)</td>
<td>-5.05 (-10.4 to 0.3)</td>
</tr>
<tr>
<td>RPT</td>
<td>5.5 (-7.8 to 18.8)</td>
<td>0.5 (-12.9 to 13.9)</td>
<td>-5.0 (-19.0 to 9.1)</td>
</tr>
<tr>
<td>75% RPT</td>
<td>4.7 (-6.3 to 15.6)</td>
<td>-0.3 (-11.3 to 10.7)</td>
<td>-5.0 (-16.4 to 6.4)</td>
</tr>
</tbody>
</table>

Values are means [95% confidence interval (CI)]. Data are shown as mean differences between IBS patients and controls for visits 1, 2, and 3. There were no significant differences in rectal pain threshold (RPT) and rectal sensory threshold (RST) between visits 1, 2, and 3 in controls and IBS patients. Repeated-measures ANOVA was used for statistical analysis on participants who attended all 3 visits.

Pain and Sensory Thresholds

RST and RPT. Controls. At visits 1, 2, and 3, RST [means (95% CI)] were 18.1 mA (15.2–20.9), 14.7 mA (12.8–16.7), and 15.8 mA (12.1–19.5), respectively, and RPTs were 70.2 mA (60.2–80.2), 71.61 mA (61.5–81.7), and 79.5 mA (69.3–89.6), respectively. Mean differences between visits 1, 2, and 3 are shown in Table 2. There were no statistical differences between visits 1, 2, and 3.

IBS patients. At visits 1, 2, and 3, RSTs were 18.7 mA (14.1–23.3), 21.3 mA (17.2–25.4), and 16.2 mA (12.6–19.9), respectively, and RPTs were 57.7 mA (48.5–66.9), 63.2 mA (53.2–73.2), and 58.2 mA (47.9–68.5), respectively. There were no significant differences between visits 1, 2, and 3 for the IBS patients (Table 2).

75% RPT. For 75% RPT, there were no differences between visits 1, 2, and 3 for the controls and IBS patients (data not shown).

IBS patients vs. controls. At visit 2, RST [mean (95% CI)] was 6.6 mA (1.4–11.7) lower for IBS patients than controls (P = 0.01; Fig. 2A). At visit 3, RPTs were significantly different between the IBS patients and controls, with a difference of −21.2 (−36.0 to −6.5) (P < 0.01; Fig. 2B). The mean 3-day RST was 16.2 mA (14.6–17.9) for controls and 18.8 mA (16.4–21.1) for the IBS patients; the difference was not significant. There was, however, a significant difference in RPT: 73.6 mA (68–79.2) for controls and 59.7 mA (54.2–65.1) for IBS patients (P < 0.001).

CEPs

CEP peak latency in controls. At visit 1, peak latencies were 73.5 ms (65.9–81.1) for P1, 119.9 ms (110.8–128.9) for N1, 224.2 ms (211.9–236.5) for P2, 397.4 ms (376.1–418.8) for N2, and 633.0 ms (589.4–676.6) for N3. The differences between visits 1, 2, and 3 are shown in Table 3. Only P2 latency was significantly different between visits 1, 2, and 3 (P = 0.04). P1 latency values for each subject at visits 1, 2, and 3 are shown in Fig. 3A.

CEP peak latencies in IBS patients. At visit 1, peak latencies [mean (95% CI)] were 7.5 µs (5.5–9.6) for P1, 19.4 µs (14.5–24.2) for N1, 19.6 µs (15.5–23.7) for P2, and 7.9 µs (6.1–9.7) for N2. There was no statistically significant difference between the 3-day measurements (Table 3).

CEP peak latency in IBS patients. At visit 1, peak latencies [mean (95% CI)] were 96.4 ms (86.6–106.1) for P1, 138.8 ms (126.1–151.4) for N1, 239.2 ms (222.9–255.5) for P2, 405.8 ms (382.9–428.7) for N2, and 633.0 ms (589.4–676.6) for N3. Differences in latency between the 3-day measurements (Table 3).

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Fig. 2. Rectal sensory threshold (RST) and rectal pain threshold (RPT) for control subjects and patients with irritable bowel syndrome (IBS). A: RST was significantly greater [by 6.6 mA (95% CI 1.4–11.7)] for IBS patients than controls at visit 2. *P = 0.01. B: RPT was significantly less [by −21.2 mA (−36.0 to −6.5)] in IBS patients than controls at visit 3. **P < 0.01 (1-way ANOVA).

ms (375.6–436.0) for N2, and 674.3 ms (630.0–718.5) for N3. Differences between visits 1, 2, and 3 are shown in Table 4; the only significant difference between visits 1, 2, and 3 was for N2 latency (P = 0.04). Individual P1 latency values for visits 1, 2, and 3 are shown in Fig. 3B.

CEP peak amplitude in IBS patients. At visit 1, peak amplitude [mean (95% CI)] was 5.1 μV (3.4–6.9) for P1, 13.3 μV (9.5–17.0) for N1, 12.5 μV (9.4–15.7) for P2, and 5.3 μV (4.1–6.5) for N2. For P1, N1, and P2 amplitudes, there was no intervisit variation; N2 amplitude showed a significant difference between visits 1, 2, and 3 (P = 0.02; Table 4).

Comparative Profiles of IBS Patients and Controls

CEPs characteristics. Latency. Mean 3-day latencies [means (95% CI)] were longer for the IBS patients than controls for P1, N1, and P2 (P < 0.001): 101 ms (88.1–113.8) vs. 73.5 ms (69.3–77.6) for P1, 138.8 ms (130.6–147.0) vs. 116.1 ms (110.8–121.4) for N1, and 241.8 ms (232.2–251.3) vs. 219.7 ms (213.7–225.6) for P2. There was no significant difference for N2 latency between the two groups: 401.66 ms (388.1–415.3) and 388.3 ms (376.9–399.6) for IBS patients and controls, respectively (P = 0.08).

The mean differences in peak latencies between IBS patients and controls at visits 1, 2, and 3 are shown in Table S1 (see Supplemental Material for this article, available online at the Journal website). N1 latency was consistently prolonged in the IBS patients compared with controls at visits 1, 2, and 3. In addition, P1 latency was significantly longer in the IBS patients at visits 1 and 2, and P2 latency was prolonged in the IBS patients at visits 2 and 3.

Amplitude. Comparison of 3-day mean amplitudes revealed lower amplitudes [mean (95% CI)] for the IBS patients than controls for the four components (P < 0.005): 5.7 μV (4.7–6.7) and 7.7 μV (6.5–9.0) for P1, 14.2 μV (12.1–16.2) and 21.4 μV (18.4–24.5) for N1, 14.1 μV (12.3–15.9) and 22.5 μV (20.0–25.0) for P2, and 6.3 μV (5.6–7.1) and 8.5 μV (7.4–9.7) for N2 for the IBS patients and controls, respectively. When P1 amplitudes in IBS patients and controls were comparable for each visit, no significant differences were noted. N1 and P2 amplitudes were consistently lower at visits 1, 2, and 3 for the IBS patients than controls. N2 amplitude was only significantly lower at visit 1 (see Supplemental Table S1).

Psychological Profile

SSTAI-state scores [means (95% CI)] for the IBS patients were 38.9 (35.4–42.3), 37.6 (33.8–41.5), and 39.7 (34.6–44.8) for visits 1, 2, and 3, respectively. In comparison, the scores for controls were 31.9 (28.8–35.0), 28.9 (26.3–31.6), and 31.6 (26.9–36.4). For each group, there were no significant interday differences for these scores (Fig. 4A; see Supplemental Table S2).

Table 3. Mean differences between visits 1, 2, and 3 for CEP components in controls

<table>
<thead>
<tr>
<th>Latency</th>
<th>Visit 2 vs. Visit 1</th>
<th>Visit 3 vs. Visit 1</th>
<th>Visit 3 vs. Visit 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (peak 1)</td>
<td>0.6 (−9.7 to 11.0)</td>
<td>−0.8 (−11.1 to 9.6)</td>
<td>−1.4 (−12.0 to 9.2)</td>
<td>NS</td>
</tr>
<tr>
<td>N1 (peak 2)</td>
<td>−5.1 (−18.1 to 7.8)</td>
<td>−6.6 (−19.5 to 6.4)</td>
<td>−1.4 (−15.0 to 12.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>P2 (peak 3)</td>
<td>−5.3 (−19.8 to 9.3)</td>
<td>−8.7 (−23.3 to 5.9)</td>
<td>−3.4 (−16.8 to 9.9)</td>
<td>NS</td>
</tr>
<tr>
<td>N2 (peak 4)</td>
<td>−11.7 (−39.4 to 6.0)</td>
<td>−16.8 (−44.6 to 10.9)</td>
<td>−5.1 (−32.7 to 22.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P1 (peak 1)</td>
<td>0.6 (−2.5 to 3.7)</td>
<td>−0.1 (−3.1 to 3.0)</td>
<td>−0.7 (−4.0 to 2.7)</td>
<td>NS</td>
</tr>
<tr>
<td>N1 (peak 2)</td>
<td>2.7 (−4.8 to 10.1)</td>
<td>3.8 (−3.7 to 11.2)</td>
<td>1.1 (−7.0 to 9.2)</td>
<td>NS</td>
</tr>
<tr>
<td>P2 (peak 3)</td>
<td>3.5 (−2.5 to 9.5)</td>
<td>5.6 (−0.4 to 11.5)</td>
<td>2.0 (−4.3 to 8.4)</td>
<td>NS</td>
</tr>
<tr>
<td>N2 (peak 4)</td>
<td>0.5 (−2.4 to 3.4)</td>
<td>1.5 (−1.4 to 4.4)</td>
<td>1.0 (−2.2 to 4.2)</td>
<td>NS</td>
</tr>
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</table>

Values are means (95% CI); n = 21. Five cortical evoked potentials (CEPs) were observed. There were no significant differences between amplitude components at visits 1, 2, and 3. Repeated-measures ANOVA was used for statistical analysis on participants who attended all 3 visits (n = 19). There was a significant difference in P2 latency between visits (P = 0.04). NS, not significant.
SSTAI-trait scores [means (95% CI)] for the IBS patients were 44.5 (40.7–48.3), 40.7 (36.7–44.7), and 44.0 (39.3–48.7) at visits 1, 2, and 3, respectively. Comparative scores for controls were 33.9 (30.8–37.0), 32.6 (29.3–35.9), and 32.2 (28.6–35.8) (Fig. 4B). There were no differences in the scores at visits 1, 2, and 3 for each group (see Supplemental Table S2).

**IBS Phenotypes: Subgroup Analysis**

**Low (≤50 mA) RPT.** In 12 IBS patients, mean 3-day RPT was ≤50 mA. P1 latency was >106 ms in four of these IBS patients and ≤106 ms in eight IBS patients. Eight of these IBS patients had visceral hypersensitivity, as defined by RPT <50 mA, with a normal-latency P1 ≤106 ms, and four showed features of hypervigilance, with high pain thresholds (≤50 mA) and high P1 latency (>106 ms; Figs. 5 and 6).

**Normal (>50 mA) RPT.** A 3-day mean pain threshold >50 mA was observed in 21 IBS patients. Five of these IBS patients exhibited prolonged (>106 ms) latency, and 16 exhibited normal latency. Hyposensitivity, as defined by high or normal pain threshold >50 mA and prolonged P1 latency >106 ms, was therefore noted in five IBS patients. The greatest proportion of IBS patients (49%) showed normal RPT >50 mA and normal P1 latency (Figs. 5 and 6).

**Normal, hypervigilant, and hypersensitive profiles.** The difference between CEP, pain threshold, and anxiety state emphasizing the above profiles is shown for three subjects in Supplemental Fig. S1.

**DISCUSSION**

This study is the first to use rectal CEP over time to examine IBS neurophysiological profiles. We show that rectal CEPs generate a reproducible, objective assessment of rectal afferent sensitivity and are consistent for interday testing in healthy controls and IBS patients. The rectal CEP recordings were also morphologically similar between visits 1, 2, and 3 for the four major components of the early primary afferent cortical response.

**CEP Interpretation**

We, and others, previously showed that the early CEP complex in response to visceral stimulation is stimulus-dependent and represents activation of the primary or secondary somatosensory cortex and insula (15). In esophageal studies, cortical activity after ~150 ms following electrical stimulation relates to brain endogenous processing of afferent information. Unlike early CEP components, which depend on the intensity of the transmitted afferent signal strength, later components appear to represent a more general arousal-type response to the stimulus. These later responses may be heightened in response to a low level of stimulation if a subject’s arousal or attention is altered and may provide an objective measure of hypervigilance.

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**Fig. 3.** Individual data points for P1 latency at visits 1, 2, and 3 for controls (A) and IBS patients (B). §P1 latency = 608.6 ms.
P1 latency represents the time interval between stimulation and activation of these sensory discriminatory brain regions. In healthy subjects, as the perceived stimulus intensity increases, there is a concurrent reduction in latency and an increase in amplitude of the CEP components (14). This provides an objective measure that correlates strongly with a subjective end point. In our previous NCCP work, we identified a group of patients expressing pain at relatively low stimulation levels but with normal P1 latency (16), indicative of afferent sensitization. A second group that also reported pain at low stimulation intensities showed prolonged-latency P1 responses. As these responses were similar to those expected when CEPs were acquired around the subject’s perception threshold, we surmised that the afferent signaling pathway was not sensitized; instead, the subjects were amplifying normal-intensity stimuli once these stimuli had reached the brain, with hypervigilance being a possible process by which this could happen. We acknowledge that stress-induced rectal hyperalgesia may also be a mechanism by which this kind of central amplification may occur, but until this is proven, the term “hypervigilance” has been used to distinguish between these two groups.

### CEP as a Cortical Sensory Processing Tool

CEP techniques have been used by neurophysiologists to investigate visual, auditory, and somatosensory pathways, leading to adaptations to study visceral sensation (3, 9). In rectal studies, different visceral stimuli, namely, electrical and mechanical modalities, generated identical CEP waveforms, except for a slight latency difference due to a 50-ms delay in the balloon inflation (11). We chose an electric modality, because it provides distinct painful stimulation, which may be more applicable in pain studies. Rectal distension responses are mediated by unmyelinated C-fibers and thinly myelinated Aδ-fibers through the pelvic nerve (19, 24, 28). A previous study...
showed shorter-than-expected CEP latencies for C-fiber stimulation (2), suggesting that electrical and mechanical rectal CEPs are mediated via A\textsubscript{\delta}-fibers.

Rectal CEP morphology exhibits some differences from CEP morphology of other gut segments; for example, P1 latency is shorter than duodenal and esophageal CEP latency (13, 17). The reason for the shorter P1 latency is unclear; further studies on conduction velocities may clarify whether this may represent faster-conducting afferent pathways conveying rectal sensation, reflecting prominence given to continence mechanisms.

CEPs have sufficient temporal resolution to discriminate between primary (stimulus-specific cortical response) and secondary (non-stimulus-specific cortical response) processing of visceral afferent activity. The latter contains endogenous pain components affected by attention, cognition, and behavior. This distinction is important, as it differentiates primary visceral hypersensitivity (increased CEP amplitude for a given stimulus) from competing delayed central contributions influenced by factors such as psychological state. Previous research on esophageal cortical sensory processing revealed that evoked potential (EP) activity up to 150 ms after stimulation represents the primary component (14–16). Activity occurring after >250 ms represents endogenous cortical activity, as observed by measurement of EP latency to anticipated esophageal stimulus (17). Studying visceral sensation with CEPs should identify whether IBS is driven by primary visceral hypersensitivity or psychological hypersensitivity.

Drewes et al. (6) took this concept of temporal resolution further by incorporating regional brain activation to map networks involved in EPs to sensory stimuli. Activity from EEGs in response to painful esophageal stimuli showed sequential activation of the thalamus, insula, cingulate, and somatosensory cortex. Using grouped CEP responses, Drewes et al. also showed subtle differences in the cingulate cortex activation region in IBS patients. Different cortical topography may explain partly the differences in latency and amplitude between various CEP components between IBS patients and controls in our study.

**CEP Components Identify Distinct IBS Phenotypes**

Individual components of rectal CEP were used to define normal population reference ranges for subgroup analysis in our IBS population to explore the prevalence of primary visceral hypersensitivity. We found that IBS represents a heterogeneous patient group with different neurophysiological profiles. Most patients with IBS (49%) had a normal pain threshold and normal sensory afferent transmission. Only a minority of IBS patients (24%) had afferent visceral hypersensitivity with pain threshold <50 mA and normal latency. A similar number showed hyposensitivity or hypervigilance, with a low pain threshold and increased latency.

Pain thresholds in IBS patients were significantly different from those in controls at visit 3. This was mainly due to habituation of RPT over time in controls, whereas values for IBS patients remained consistent. Habituation to painful stimulation is mediated via descending inhibitory mechanisms, which have been shown to be defective in IBS patients in several studies (23, 32). This might suggest that the treatment strategy for attenuating visceral afferent transmission (via analgesics) would differ from boosting endogenous pain mechanisms, for example, endogenous opioids, such as \beta-endorphin. A recent preclinical study with probiotics showed that attenuation of experimental visceral hypersensitivity was associated with upregulation of peripheral opioid receptors (30).

In previous work from our department, Murray et al. (22) demonstrated little difference in sensory thresholds between IBS patients and controls until experimental stress was applied (22). The wide range of sensory response from hyper- to hyposensitivity seen in our cohort most likely skews the sensory threshold data upward. Pain thresholds differed be-
tween controls and IBS patients only because of an increase in pain threshold in controls over time; this appears to be attenuated in some IBS patients.

We also looked at potential confounders, such as state and trait anxiety. Of the five studies that previously used SSTAI for IBS patients, only three recorded mean SSTAI-state scores with an overall mean of 47.7 (8). Only one study recorded a mean SSTAI-state score of 35.5 for a control group. The mean SSTAI-trait score was 47.2 for IBS patients (in 4 studies) and 41.2 for controls (in 2 studies) (8). The SSTAI-trait score is more time-independent than the SSTAI-state score, but both indexes showed little variation in either group over visits 1, 2, and 3. At no point did the state score drop below the case threshold of 35 in the IBS group.

Conclusions
This is the first study to validate CEP testing in IBS. Testing was reproducible and robust. Early CEP responses and pain thresholds defined distinct neurophysiological groups, supporting the concept of IBS as a heterogeneous syndrome. Local and central mechanisms are likely to contribute to each profile; defining each contribution may allow effective drug therapy. The abundant CEP data generated from this study can be used in future therapeutic studies.

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